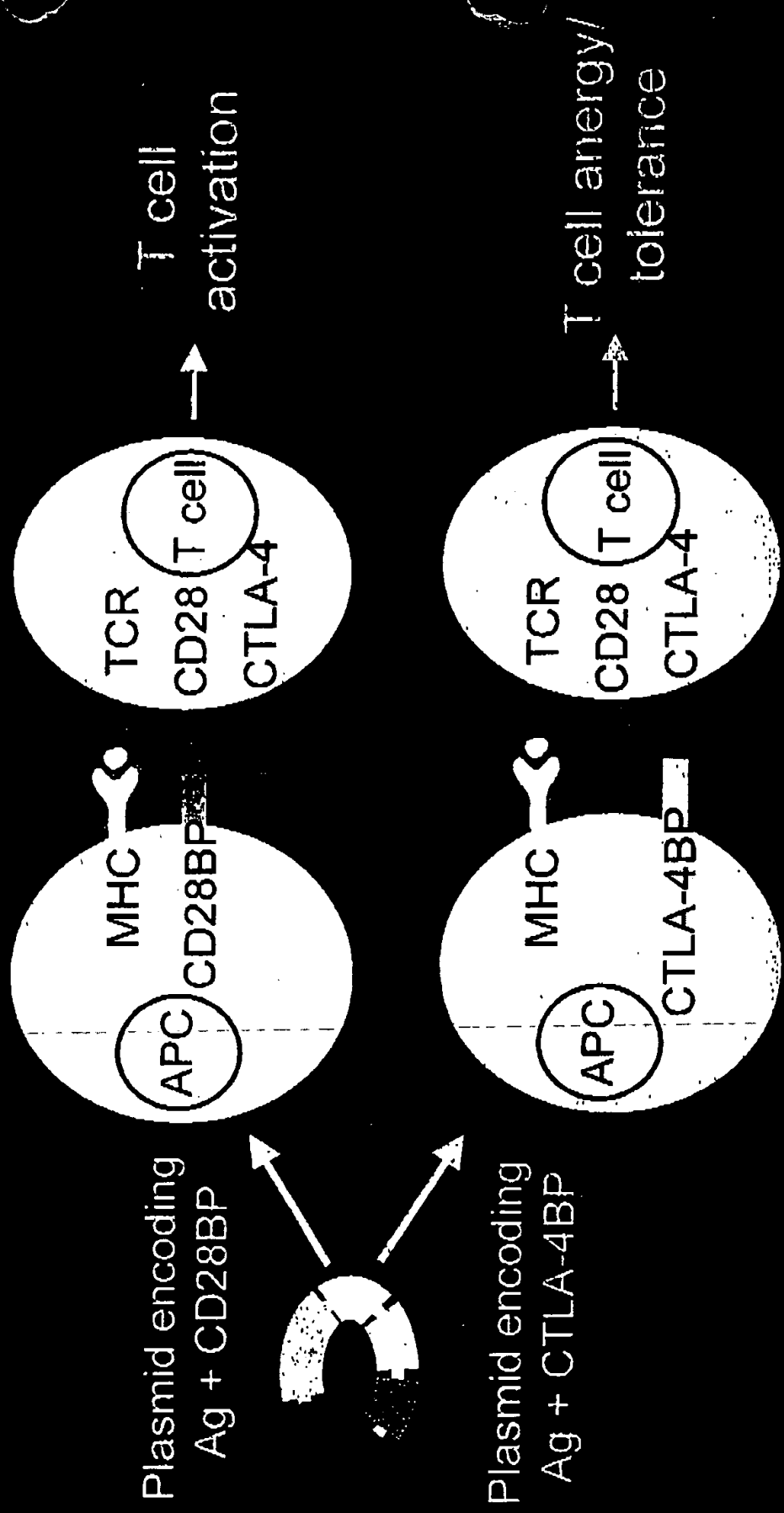


# Altered T cell Function Evolved Ligand Binding



MA YGEN

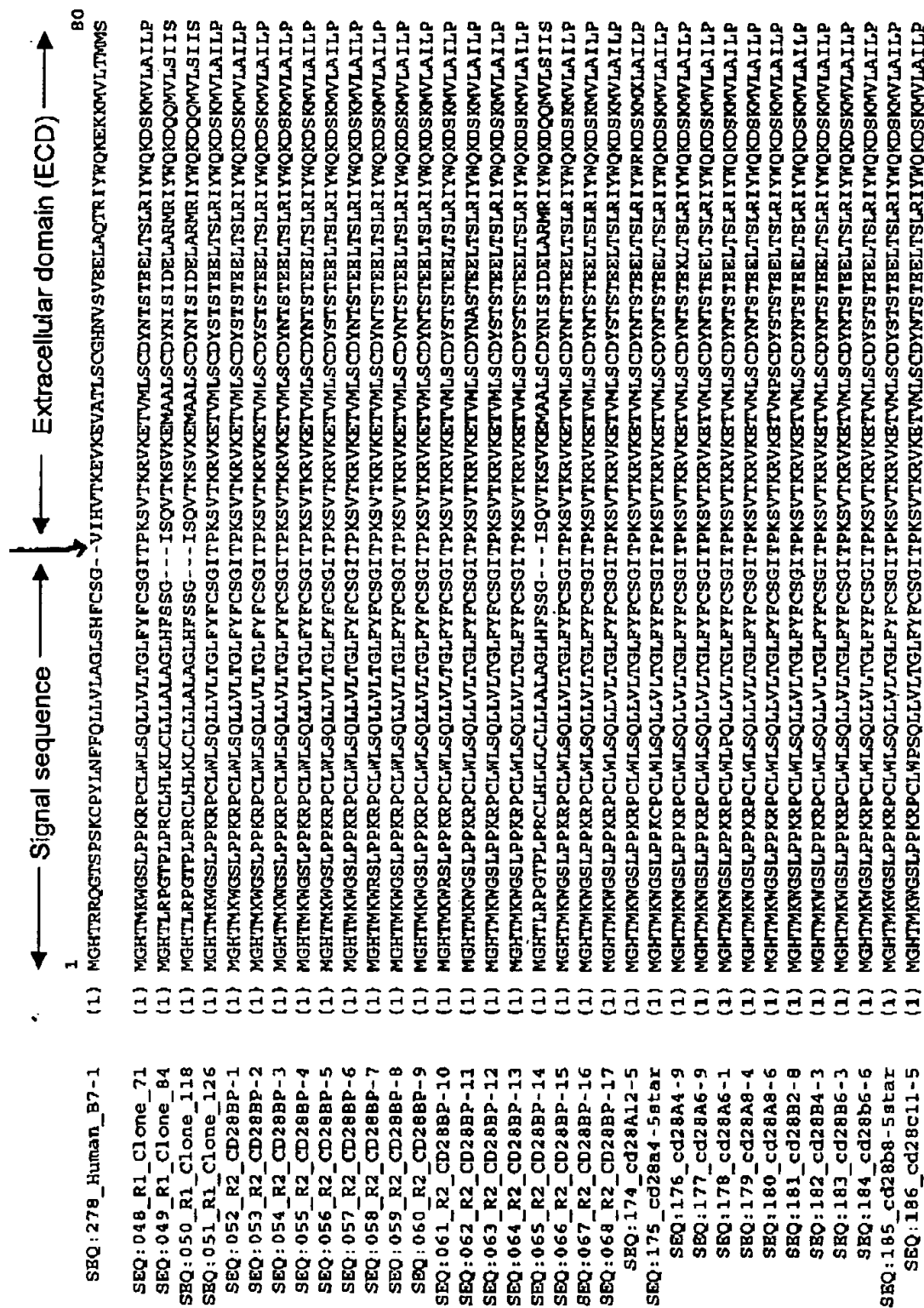
[illegible]

Fig. 2A



Fig. 2C

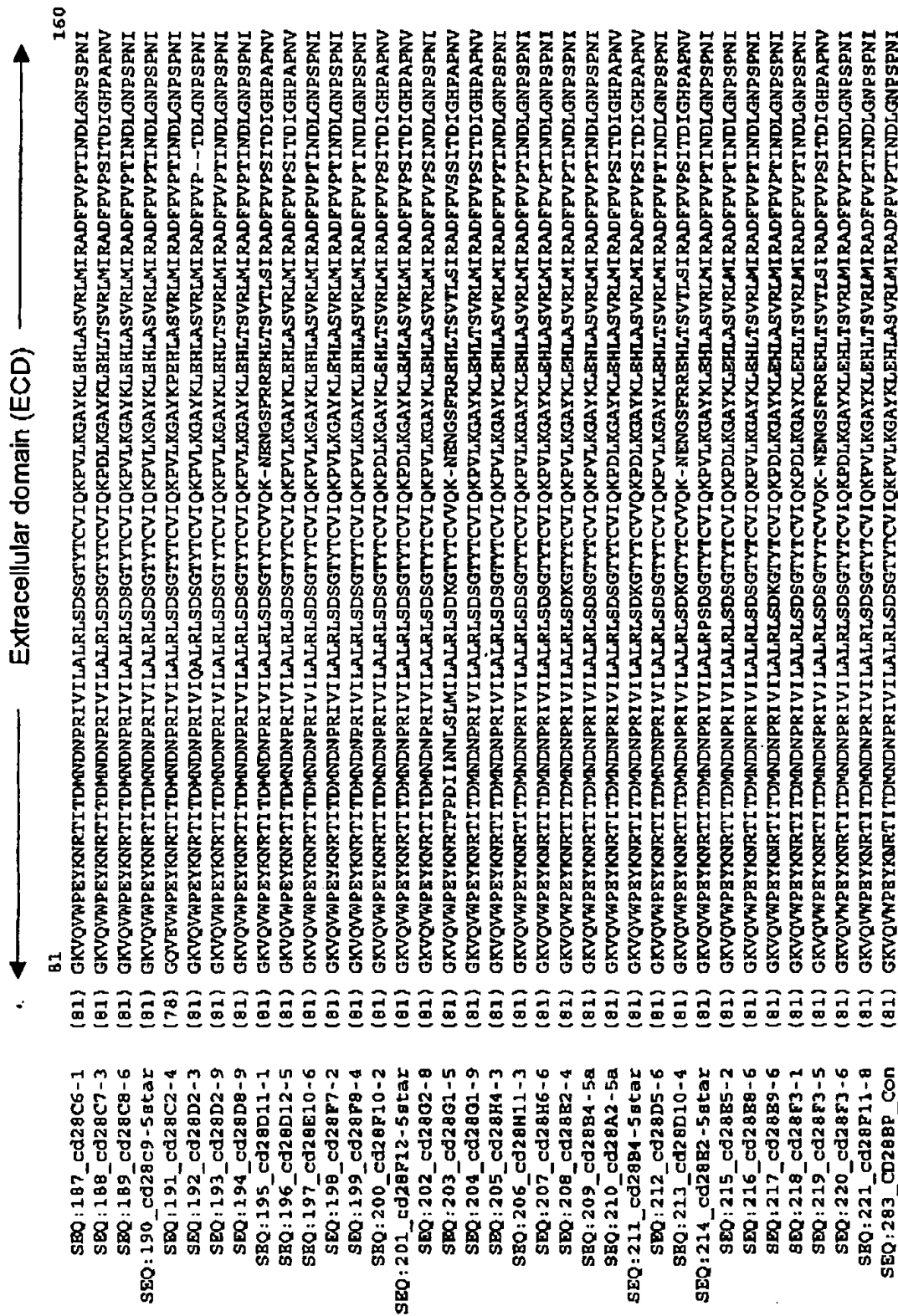


Fig. 2D

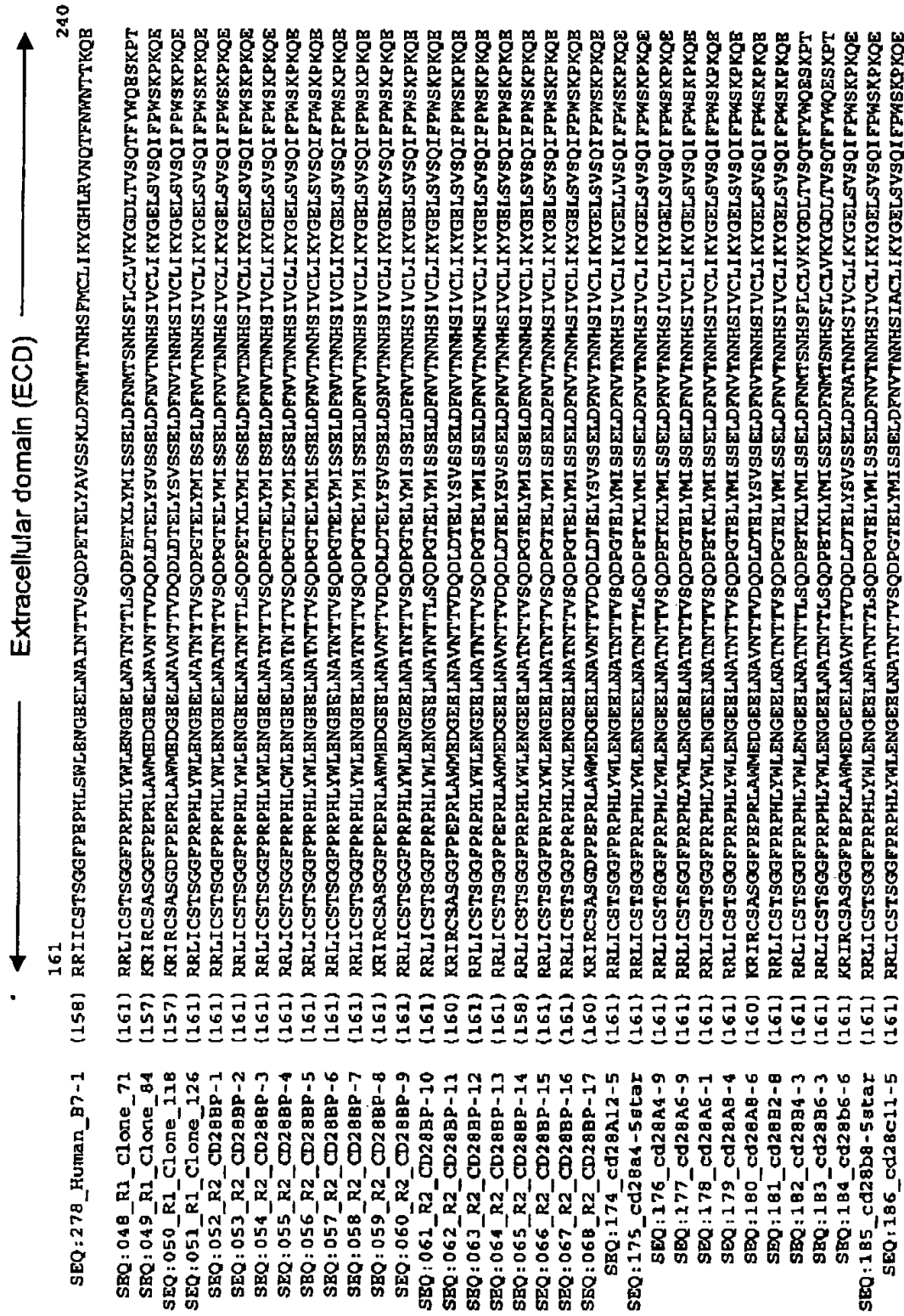


Fig. 2E

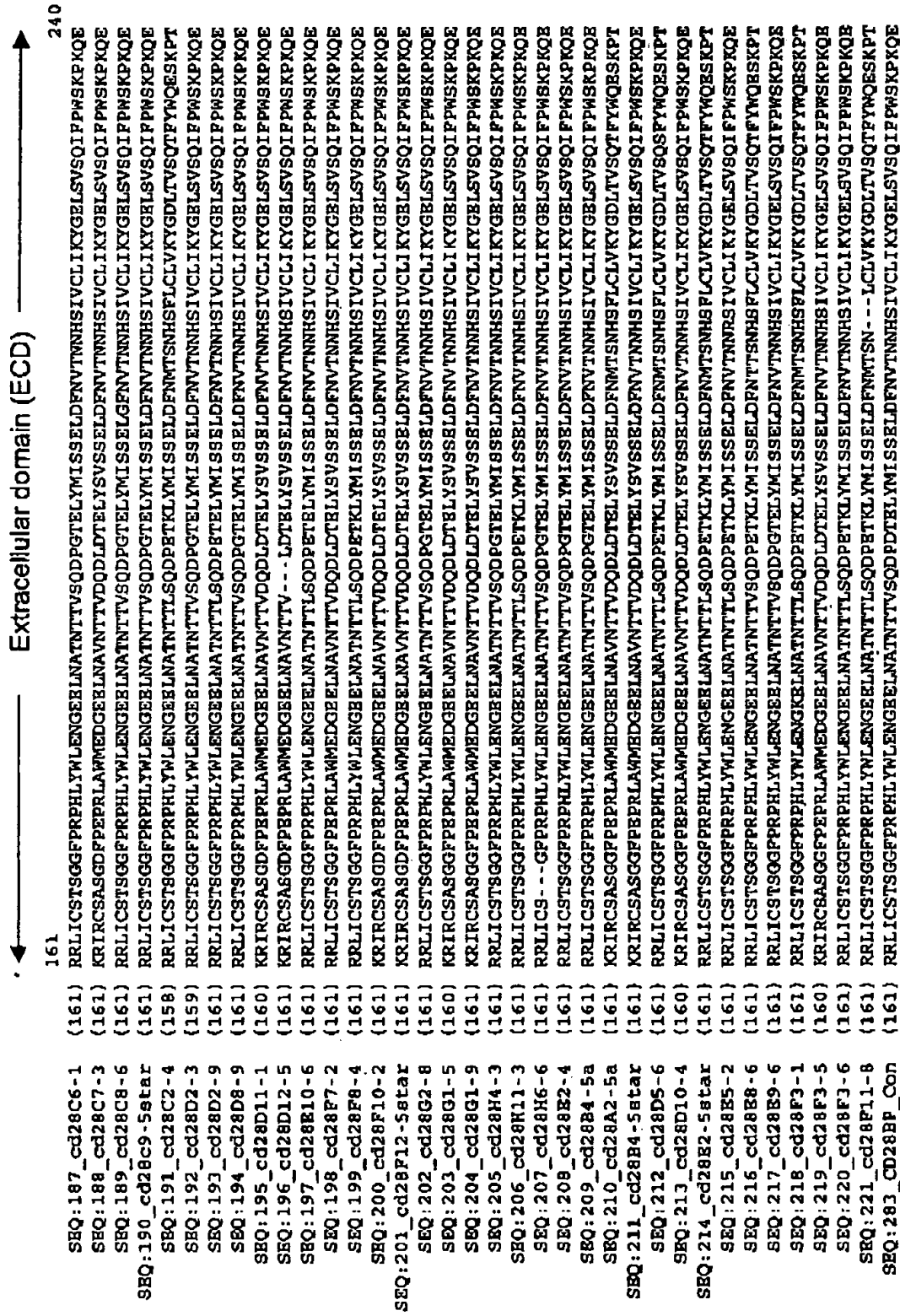


Fig. 2F

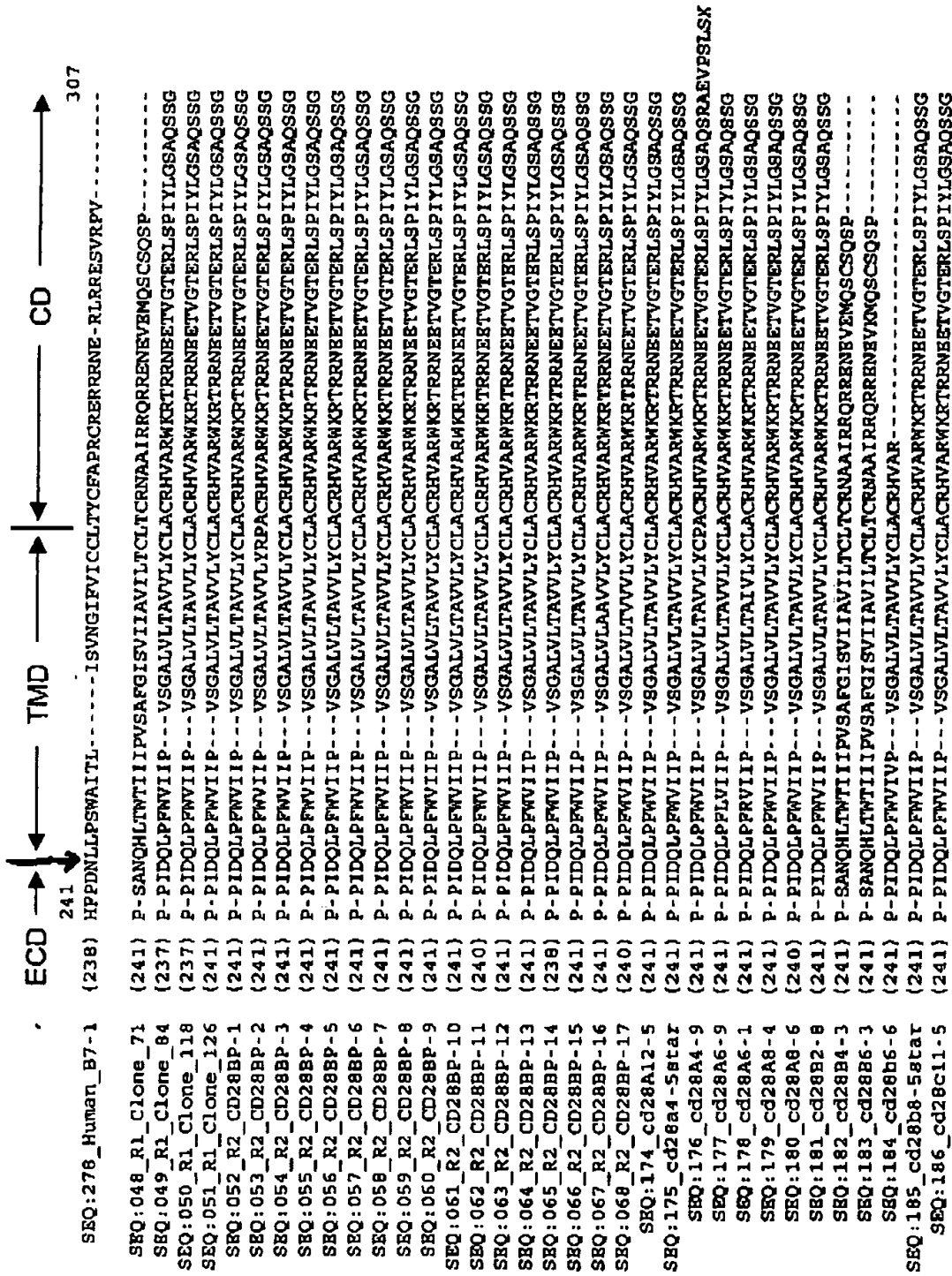


Fig. 2G



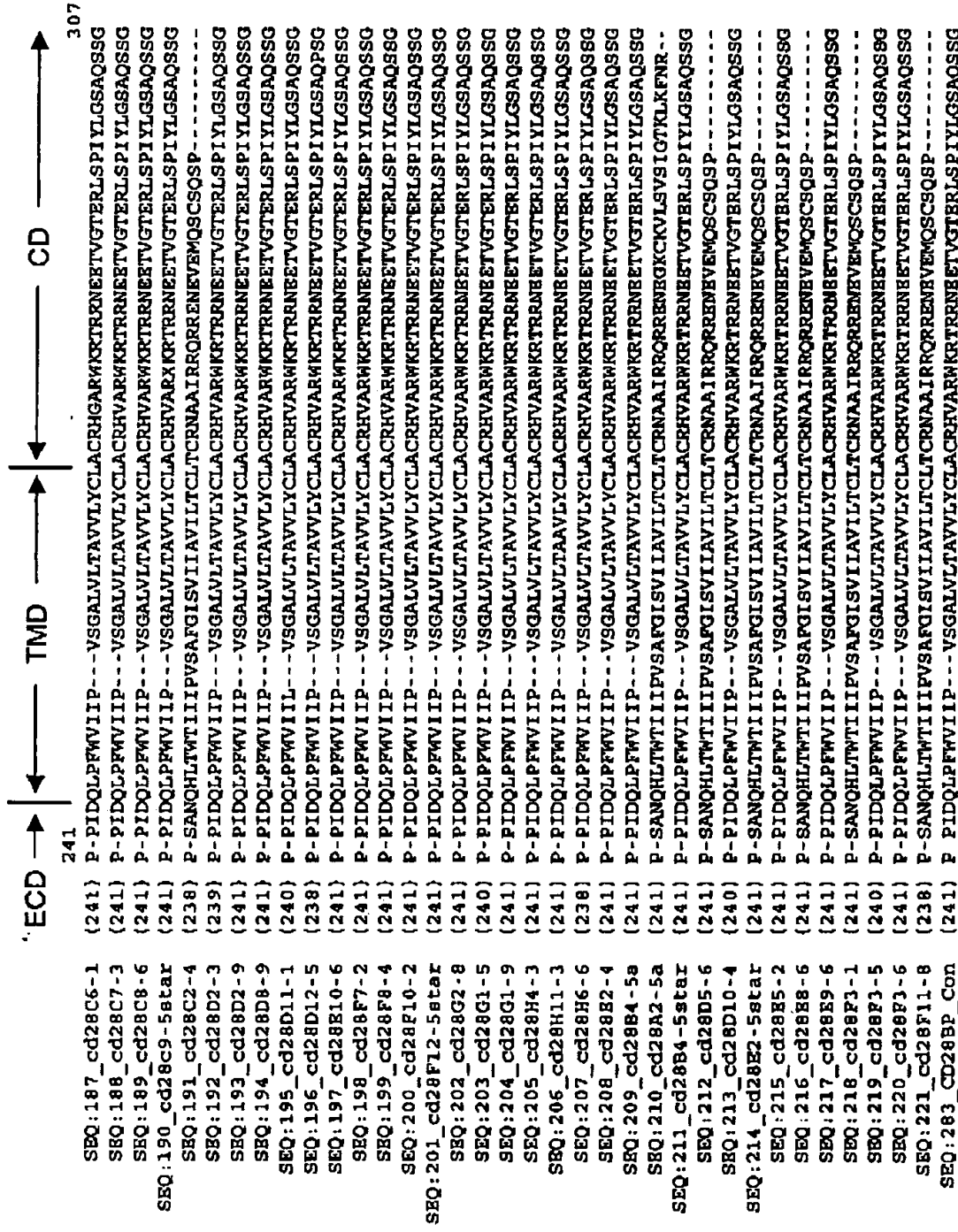
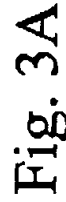


Fig. 2H

```

SEQ: 069_R1_CTLA4BP-5
SEQ: 070_R1_CTLA4BP-7
SEQ: 071_R1_CTLA4BP-11
SEQ: 072_R1_CTLA4BP-13
SEQ: 073_R1_CTLA4BP-27
SEQ: 074_R2_CTLA4BP-5x2-10c
SEQ: 075_R2_CTLA4BP-5x2-11d
SEQ: 076_R2_CTLA4BP-5x2-12F
SEQ: 077_R2_CTLA4BP-5x2-2g
SEQ: 078_R2_CTLA4BP-5x2-3c
SEQ: 079_R2_CTLA4BP-5x2-4c
SEQ: 080_R2_CTLA4BP-5x2-7b
SEQ: 081_R2_CTLA4BP-5x2-8c
SEQ: 082_R2_CTLA4BP-5x3-10ee
SEQ: 083_R2_CTLA4BP-5x3-11b
SEQ: 084_R2_CTLA4BP-5x3-6f
SEQ: 085_R2_CTLA4BP-5x4-11d
SEQ: 086_R2_CTLA4BP-5x4-12c
SEQ: 087_R2_CTLA4BP-5x4-1f
SEQ: 088_R2_CTLA4BP-5x5-2e
SEQ: 089_R2_CTLA4BP-5x5-6e
SEQ: 090_R2_CTLA4BP-5x6-9d
SEQ: 091_R2_CTLA4BP-5x8-1f
SEQ: 092_R2_CTLA4BP-5x9-12c
    SEQ: 222_ctla5x9d10
    SEQ: 223_ctla5x6f6
    SEQ: 224_ctla5x5h12
    SEQ: 225_ctla5x5c10

```



```
SEQ:226_ctla5x3e8
SEQ:227_ctla5x3c4
SEQ:228_ctla5x3c4
SEQ:229_ctla5x2h11
SEQ:230_ctla5x2d7
SEQ:231_ctla5x2b7
SEQ:232_ctla5x2b1 ns
SEQ:233_ctla5x1d7
SEQ:234_ctla5x1d7
SEQ:235_ctla2x4g9
SEQ:236_ctla2x4a6
SEQ:237_ctla2x2f3
SEQ:238_ctla2x2f12
SEQ:239_ctla2x1g8
SEQ:240_ctla2x1f10
SEQ:241_ctla2x1c9
SEQ:242_ctla2x1h12
SEQ:243_ctla2x1e2
SEQ:244_ctla2x1c4
SEQ:245_ctla2x1b12
SEQ:246_ctla2x2f1
SEQ:247_ctla5x4h1
SEQ:248_ctla5x4a1
SEQ:249_ctla5x2f3
SEQ:250_ctla5x2e12
SEQ:251_ctla2x4h11
SEQ:252_ctla2x3h2
SEQ:286_CTLA4BP3Con
```

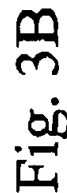


Fig. 3B

File 30

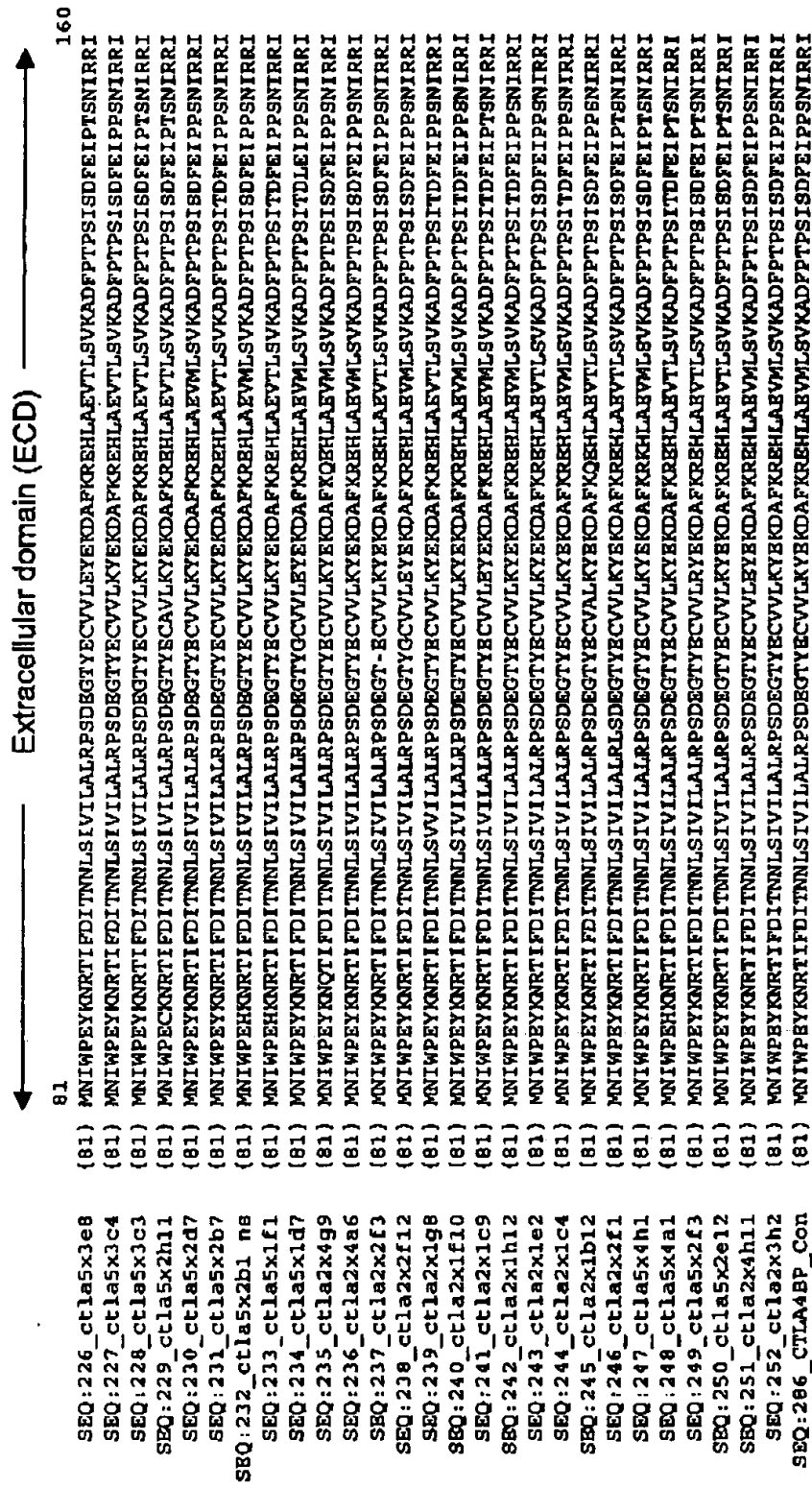


Fig. 3D

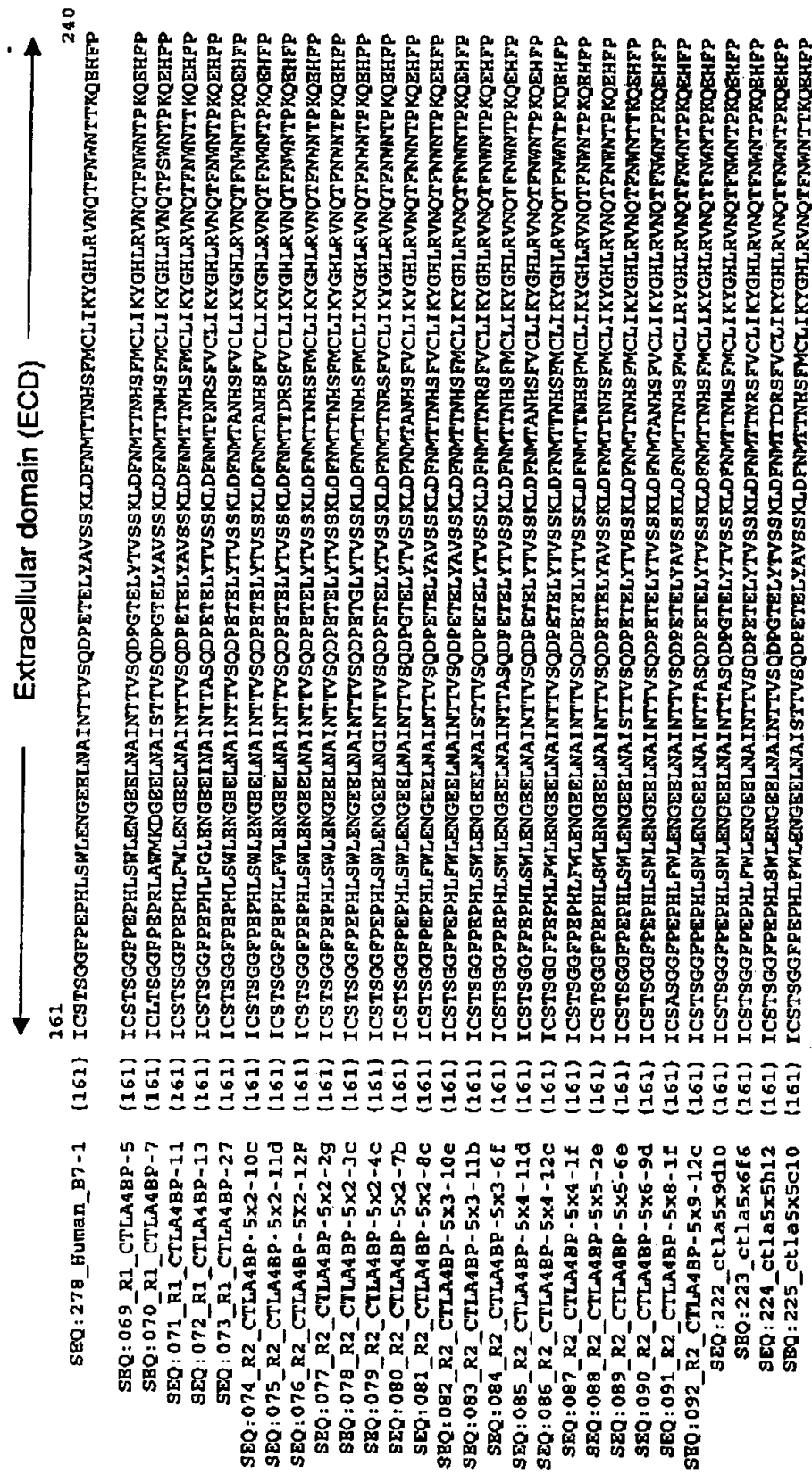


Fig. 3E

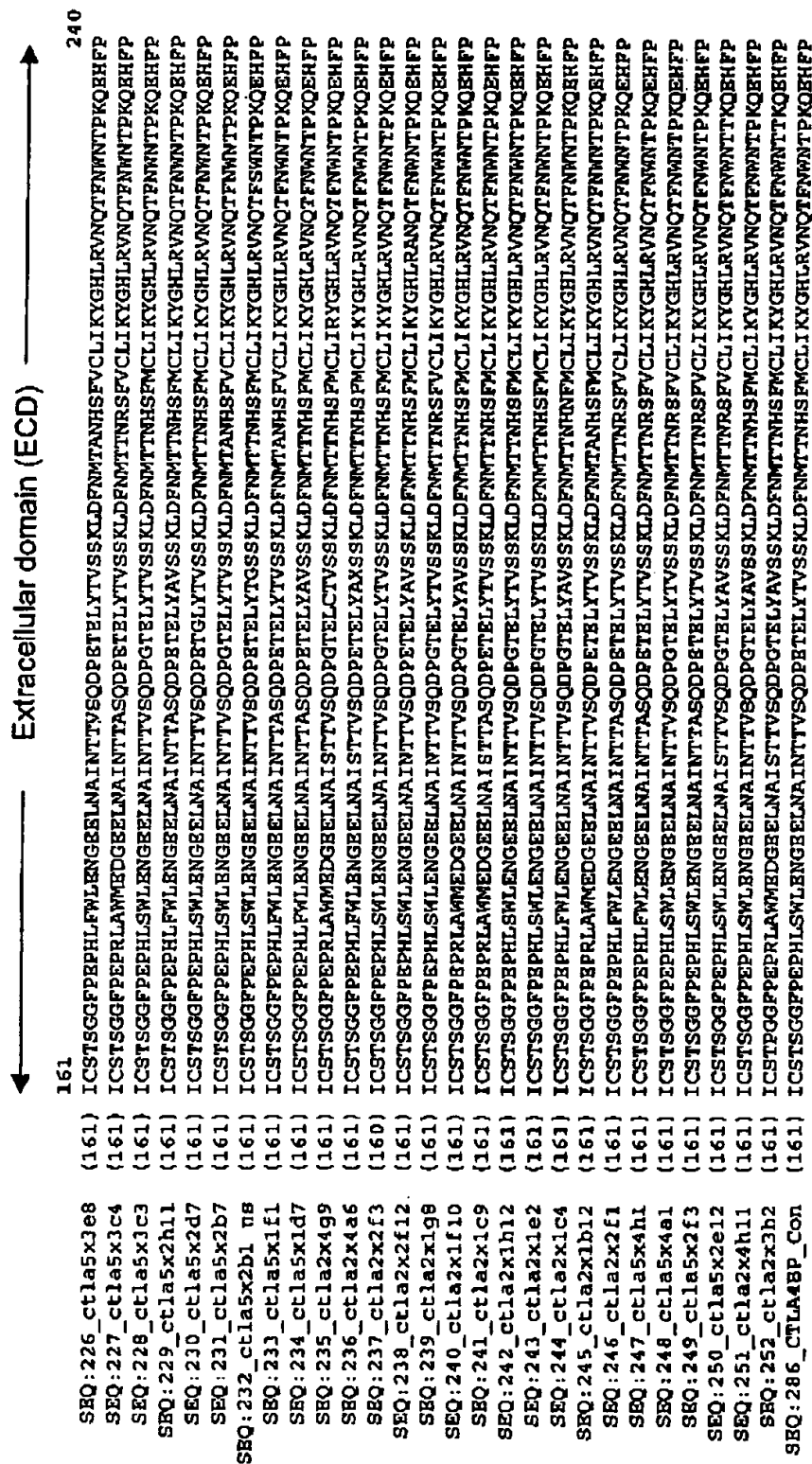


Fig. 3F

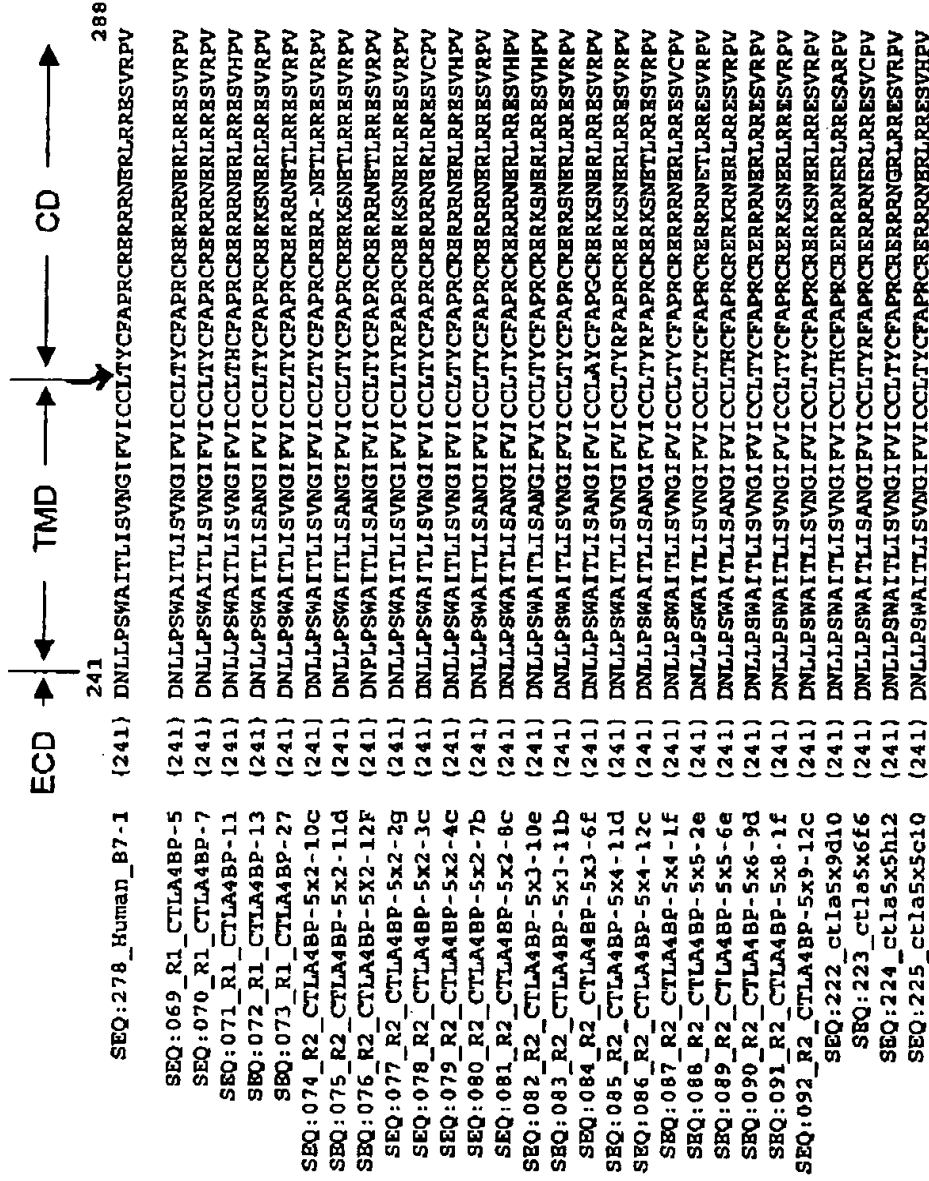


Fig. 3G



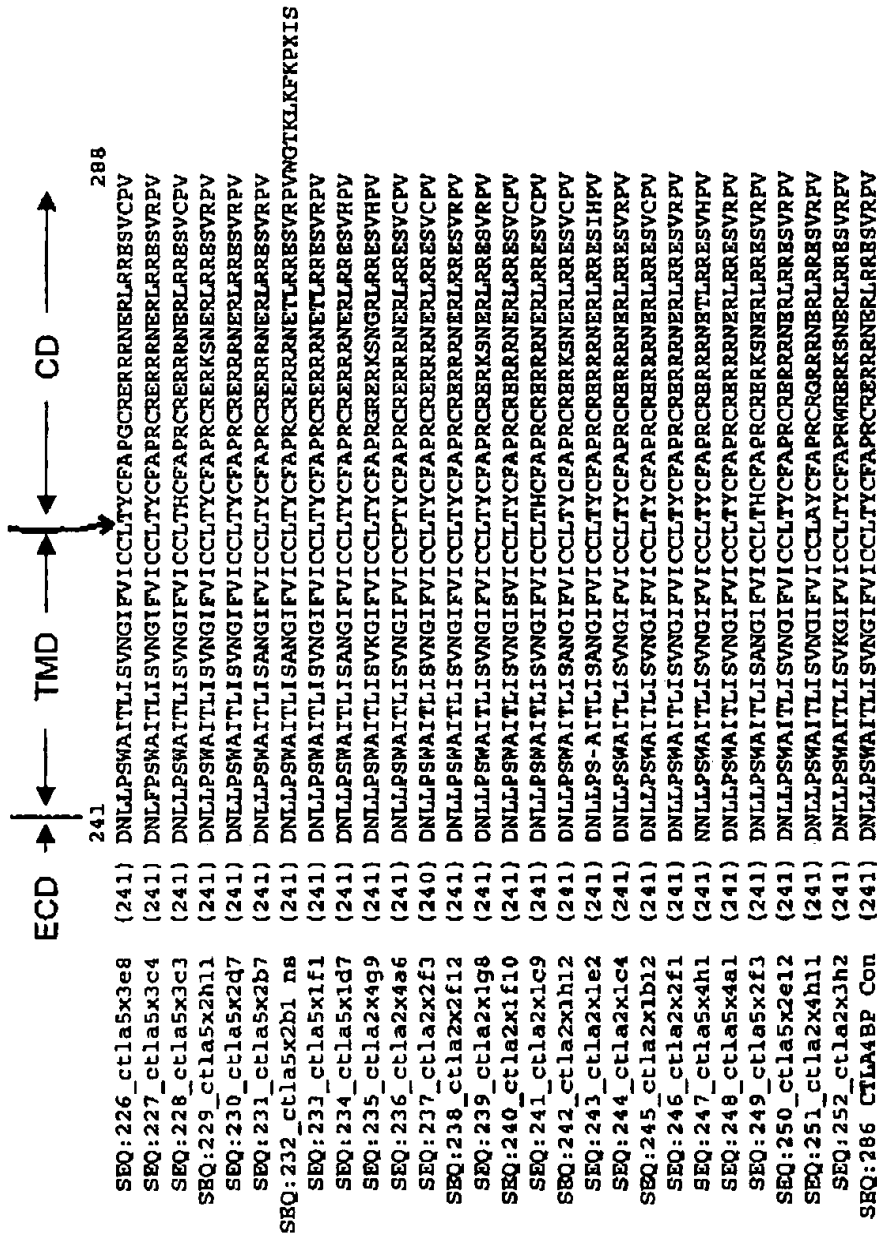


Fig. 3H

# Figures 4A-4D

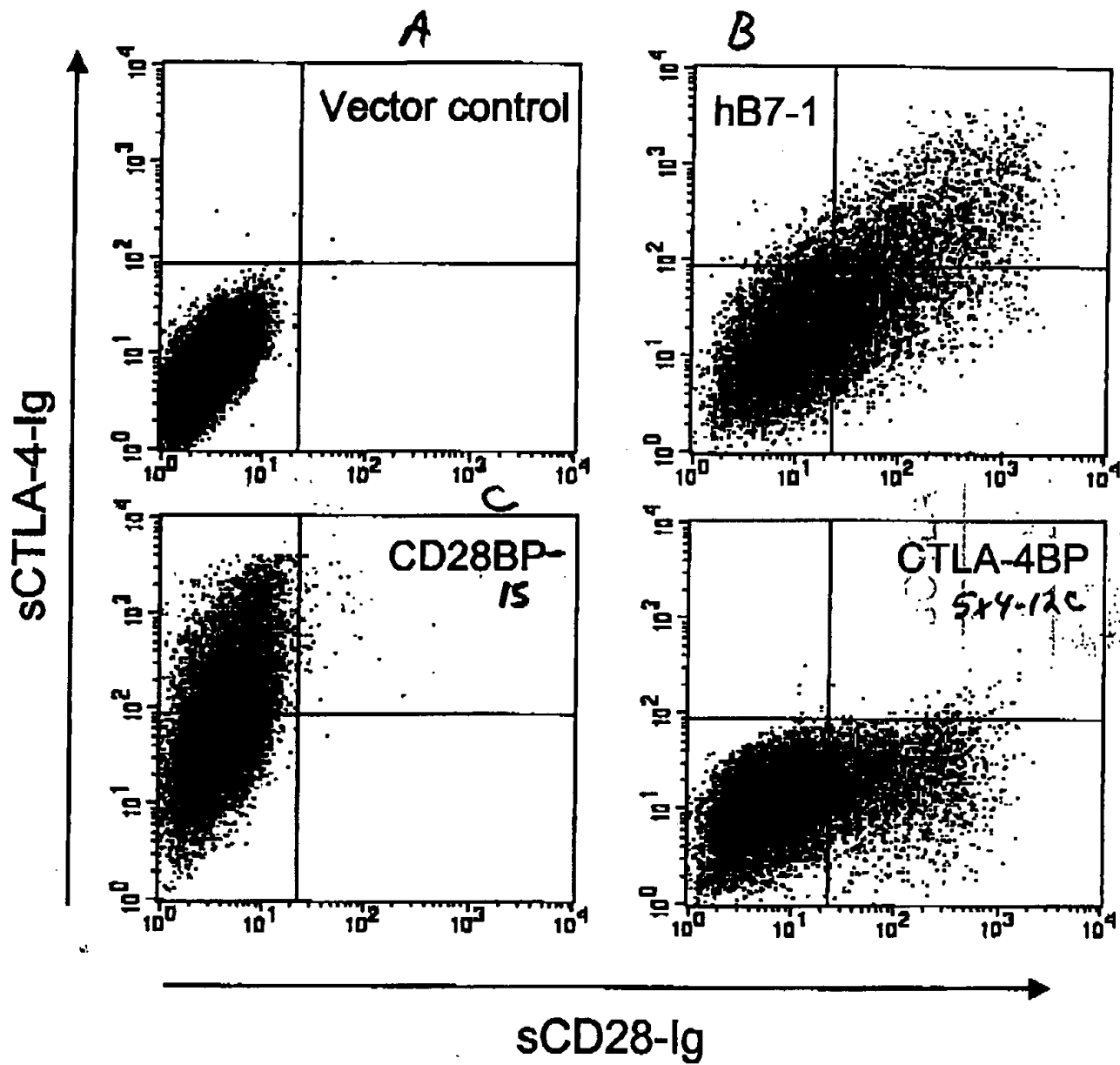
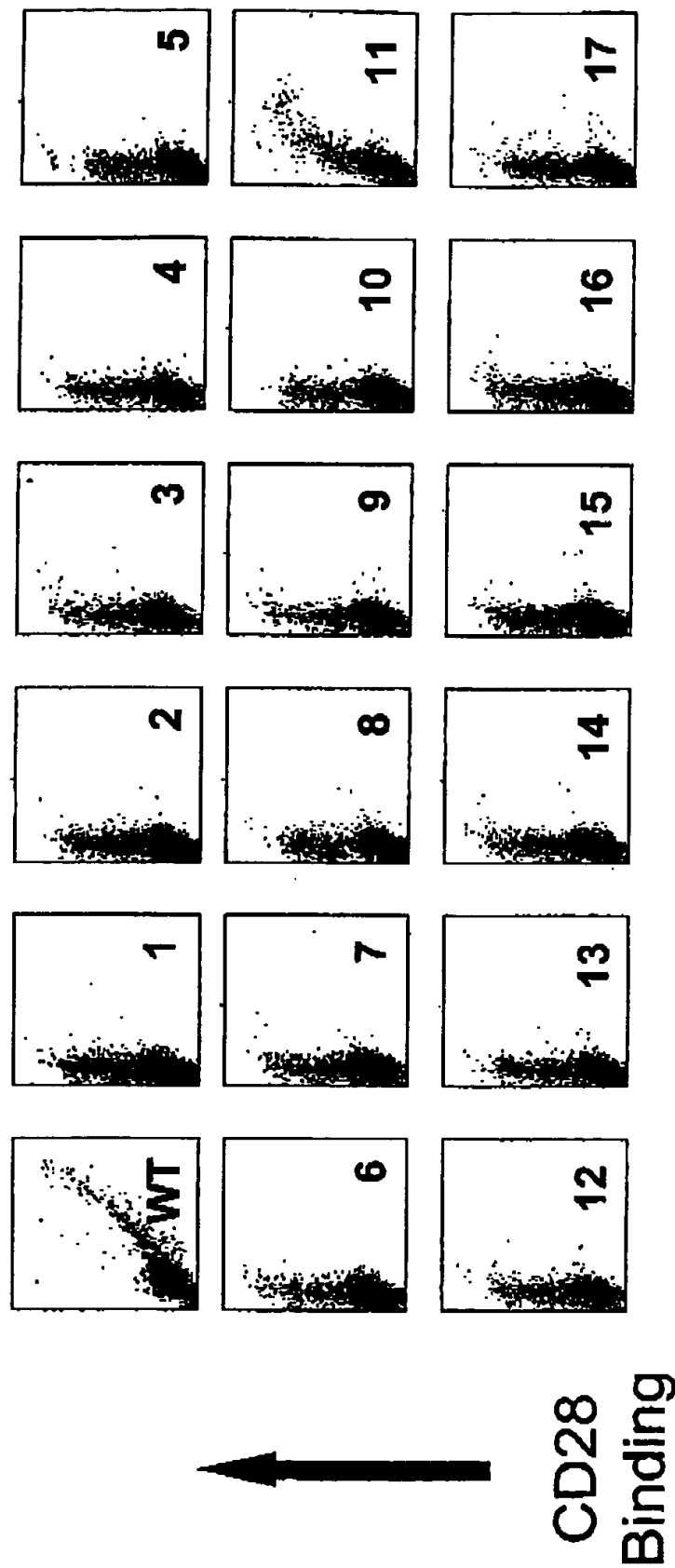


Figure 5

# CD28BP after 2<sup>nd</sup> Round of Shuffling



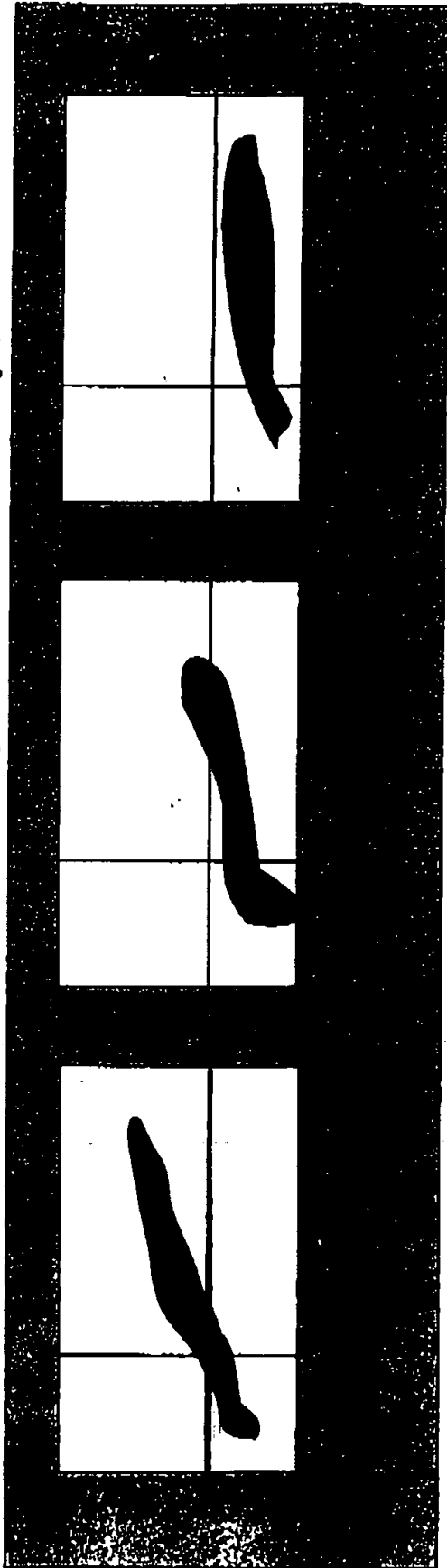
Figures A-GB.

72250-723860

A(1)

A(2)

A(3)

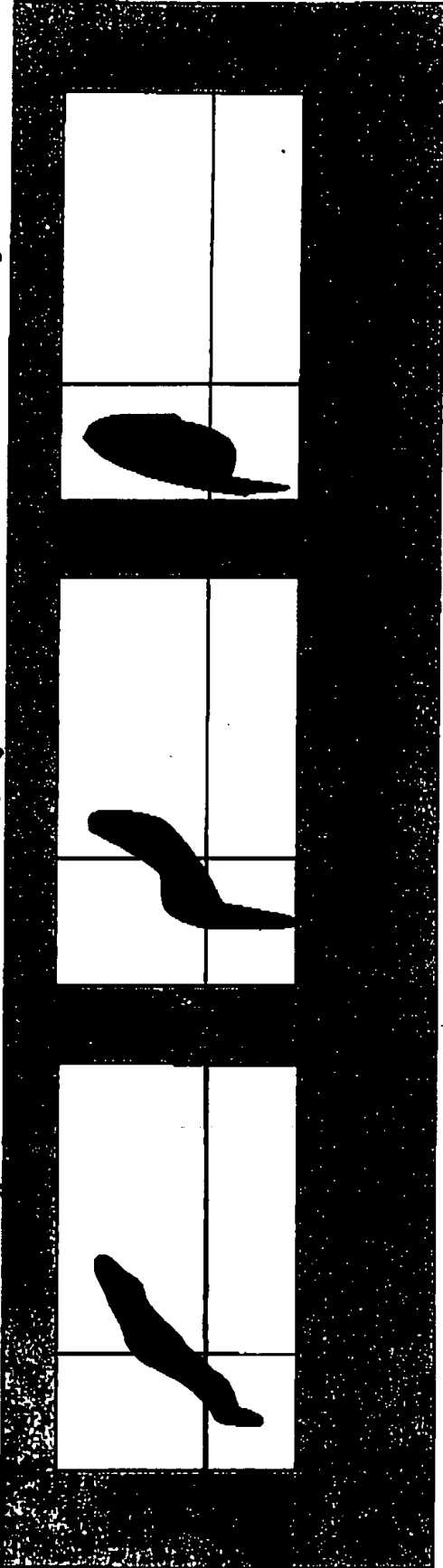


CD28-Ig Binding →

B(1) CTLA-4-Ig Binding →

B(2)

B(3)

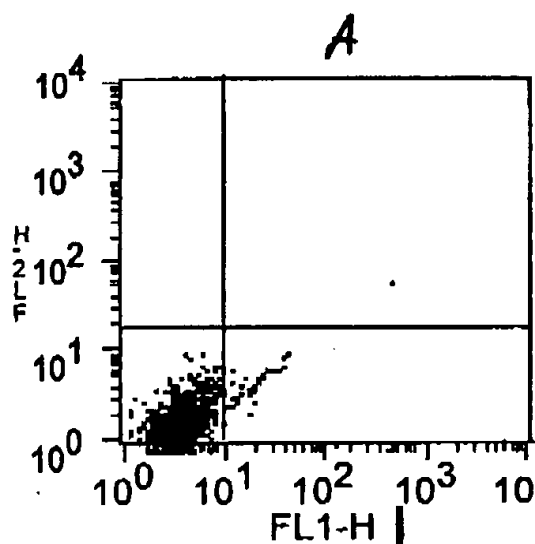


CD28-Ig Binding →

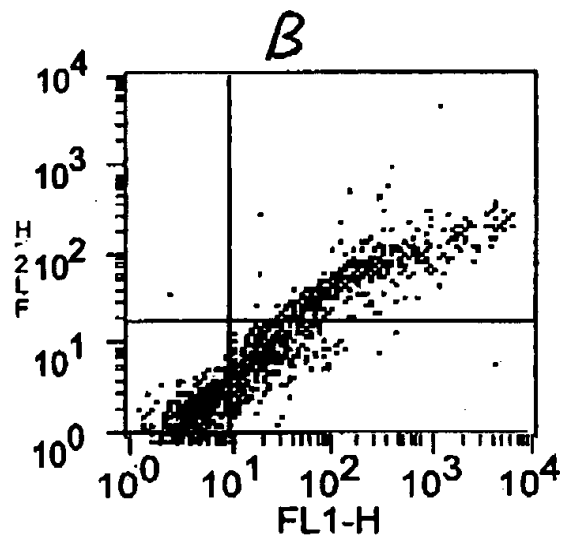
CTLA-4-Ig Binding →

Figures 1A-7D

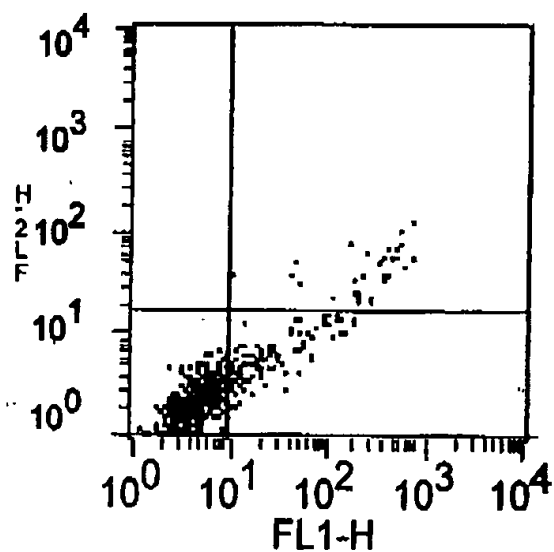
CD28 Binding (PE)



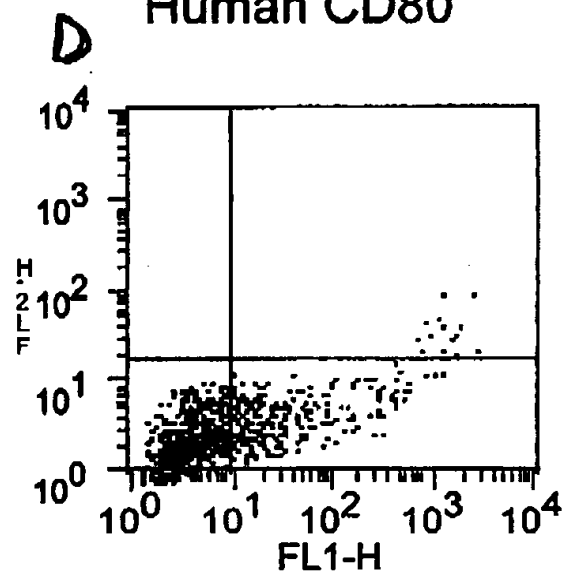
HEK 293



Human CD80



CTLA4BP R25X5-2E

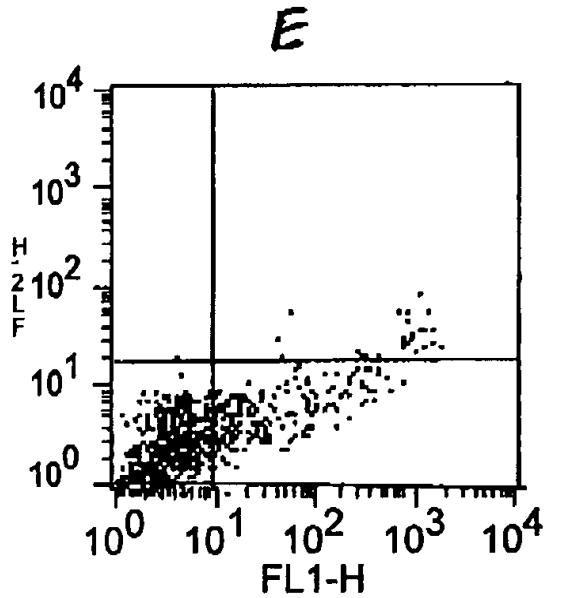


CTLA4BP R25X4-12C

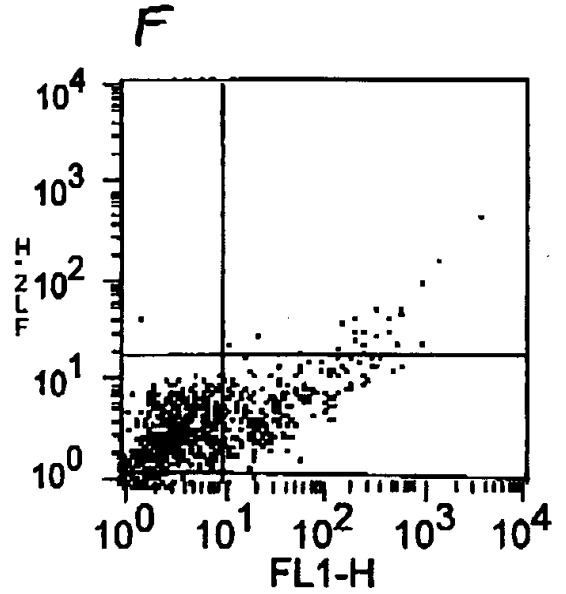
CTLA4 Binding (FITC)

Figures 1E-7H

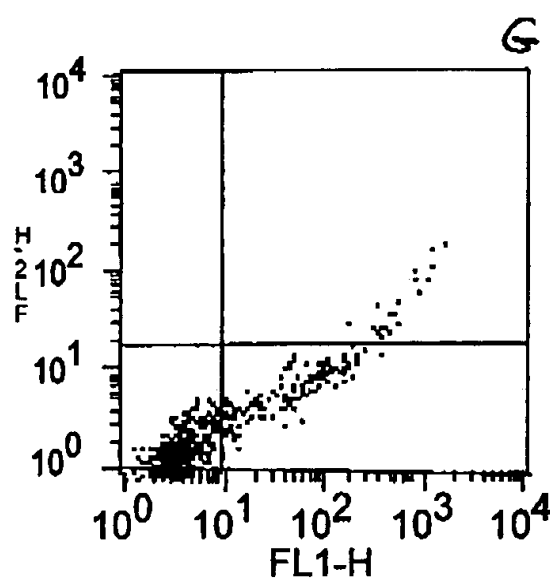
CD28 Binding (PE)



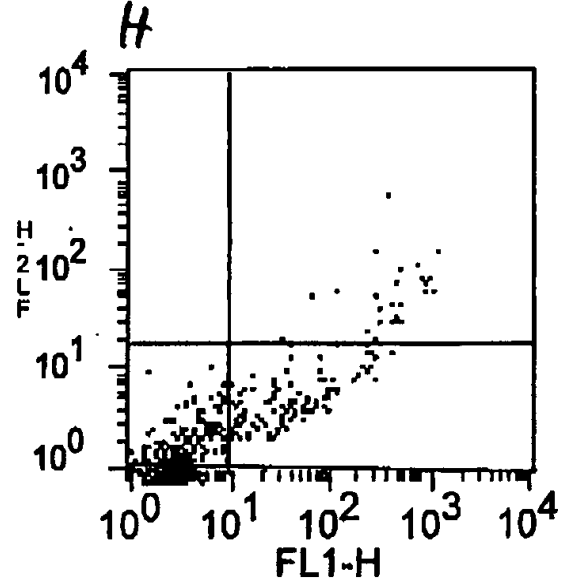
CTLA4BP R25X4-11D



CTLA4BP R25X2-8C



CTLA4BP R25X6-9D



CTLA4BP R25X8-1F

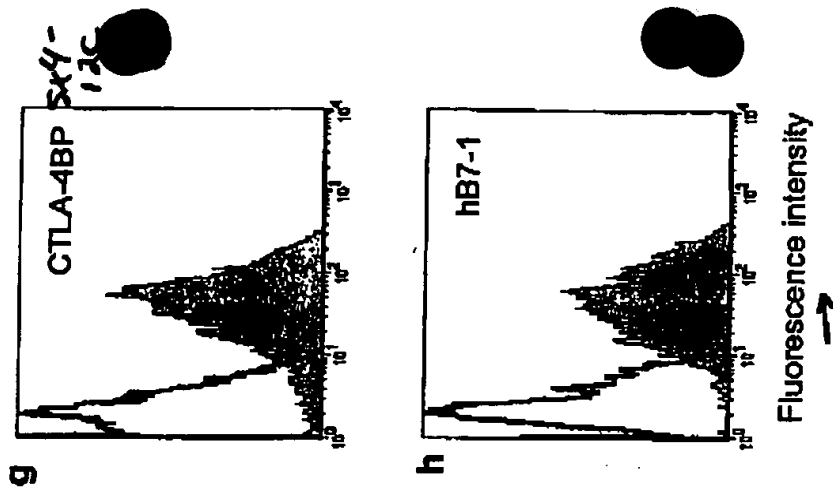
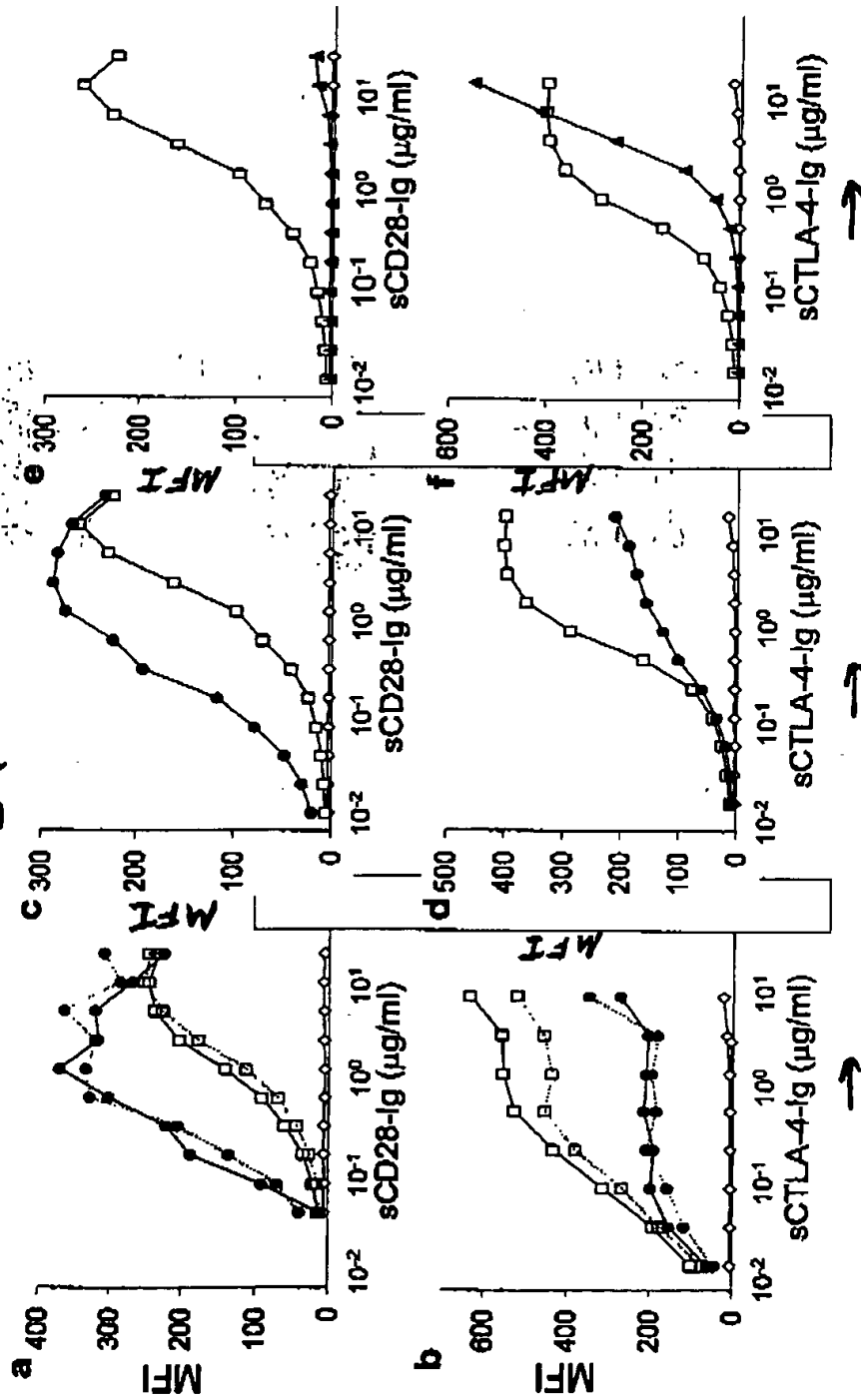
CTLA4 Binding (FITC)

a CTLA-4BP - 5x4-12c

MGHTRRQGTSPSKPYLKFFQLLVAGLSHFCSGVIHVTKVEVATLSCGHNVSVEELAQT  
 K human → baboon ← human →  
 RIHWQKEKKMVLTMMSGDMNIWPEYKNRTIFDITNLSIVILALRPSDEGTYECVVLKYEKDAF  
 human \* → rhesus/baboon \*  
 KREHLAEVMSVKADFPTPSISDFEIPPSNIRRICSTSGGFPEPHLFWLENGEELNAINTTVSQ  
 rhesus/baboon → human → baboon \*  
 DPETELYTVSSKLDENMTTNHSMCLIKYGHLRVNQTFNWTNPKQEHFPDNLPSWAITLISA  
 → rhesus  
 NGIFVICLTYRFAPRCRERKSNETLRRESVRPV  
 human → orangutan baboon → rhesus →

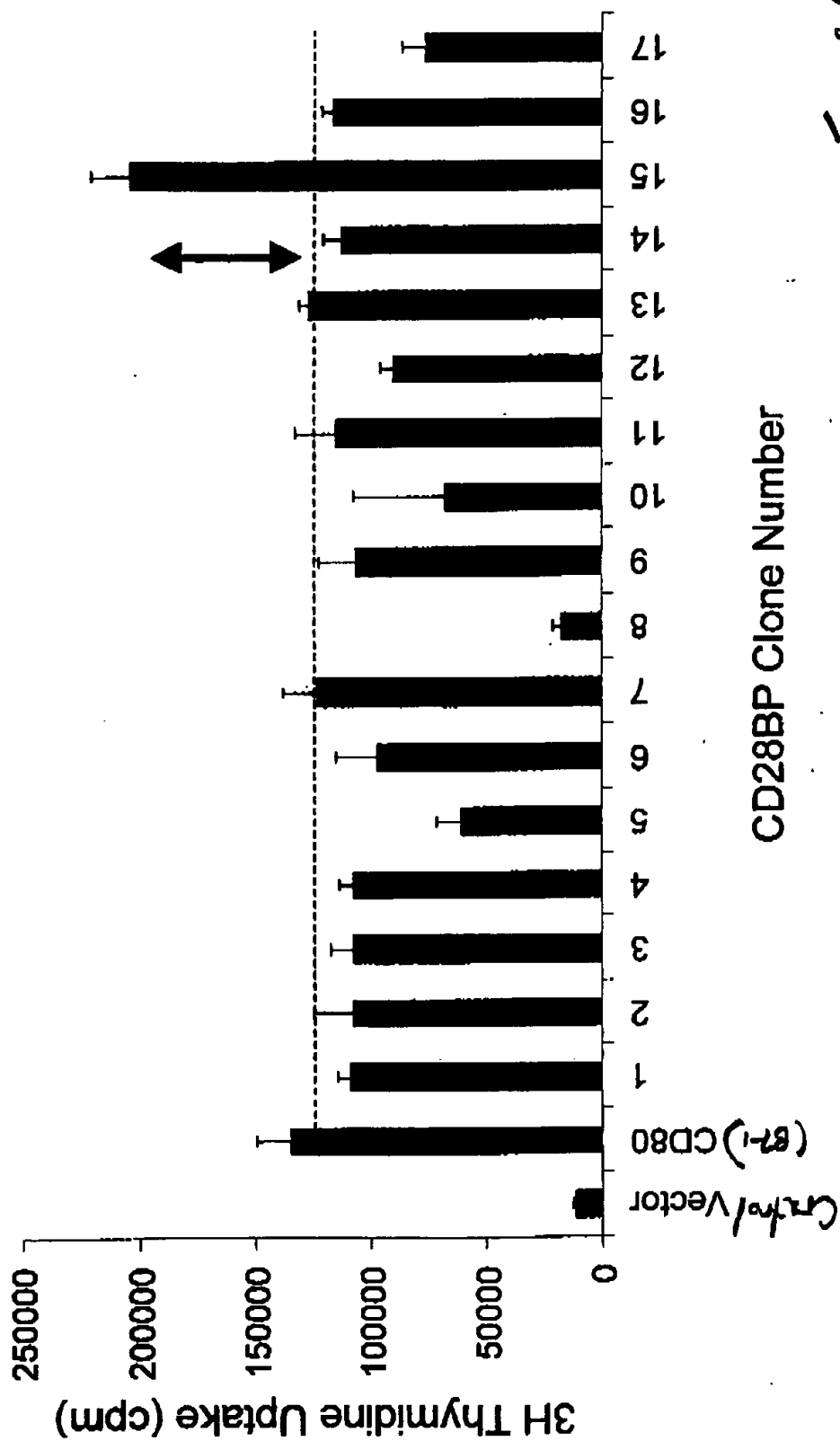
b CD28BP-15

MGHTMKWGSPPKRPCLWLSQLLVLTGLFYFCSGITPKSVTKRVKETVMLSCDYNTSTEELT  
 ← cow  
 SLRIYWQKDSKMVLAILPGKVQVWPEYKNRTITDMNDNPRIVILALRPSDSGTYTCVIQKPVLK  
 → cow  
 GAYKLEHLASVRLMIRADFPVPTINDLGNPSNIRRLICSTSGGFPRPHLYWLENGEELNATNT  
 cow → human → cow  
 TVSQDPGTLEYMISSLDENVYNNHSIVCLIKYGELSVSQIFPWSKPKQEPIDQLPFWVIPVS  
 baboon → rabbit  
 GALVLTAVLYGLACRHVARWKRRNEETVGTERTLSPIYLGSAQSSG  
 .....  
 human rhesus/baboon  
 orangutan cow  
 rhesus rabbit  
 baboon cow



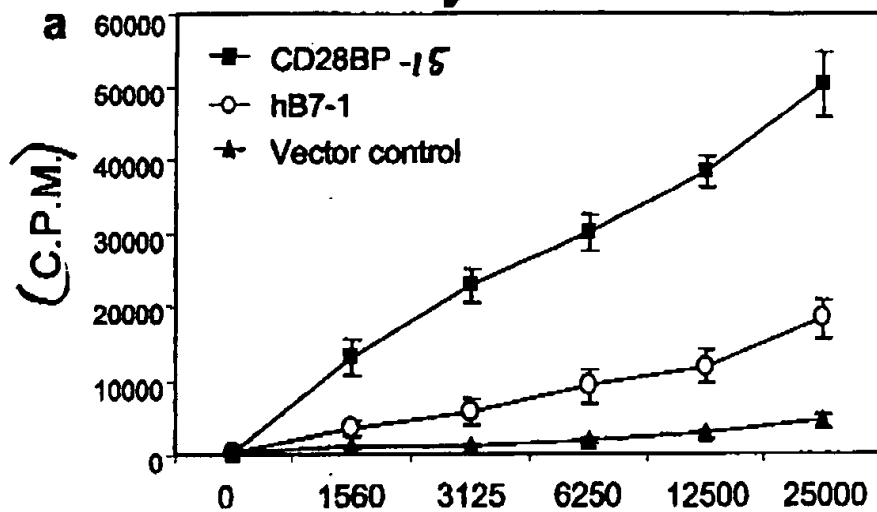
Figures 9A-9H





Figures 11A-11C

<sup>3</sup>H Thymidine Uptake



<sup>3</sup>H Thymidine Uptake

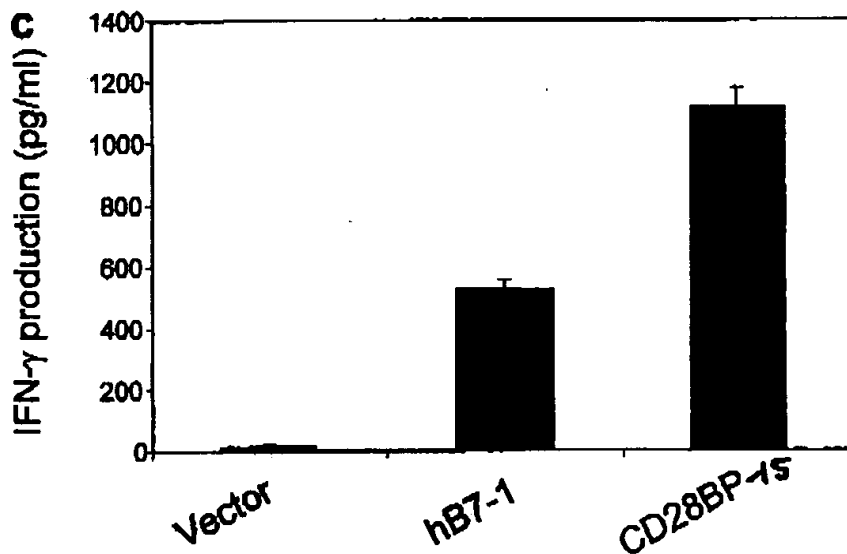
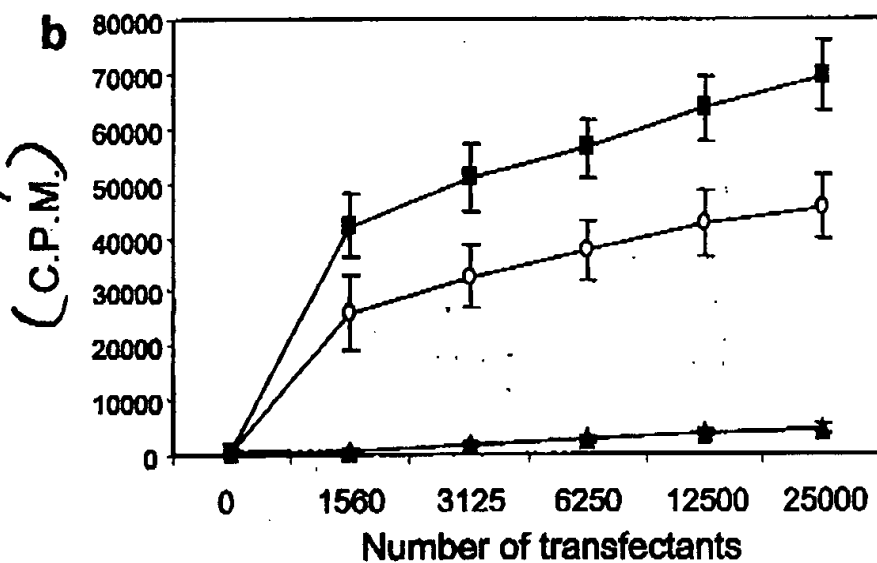
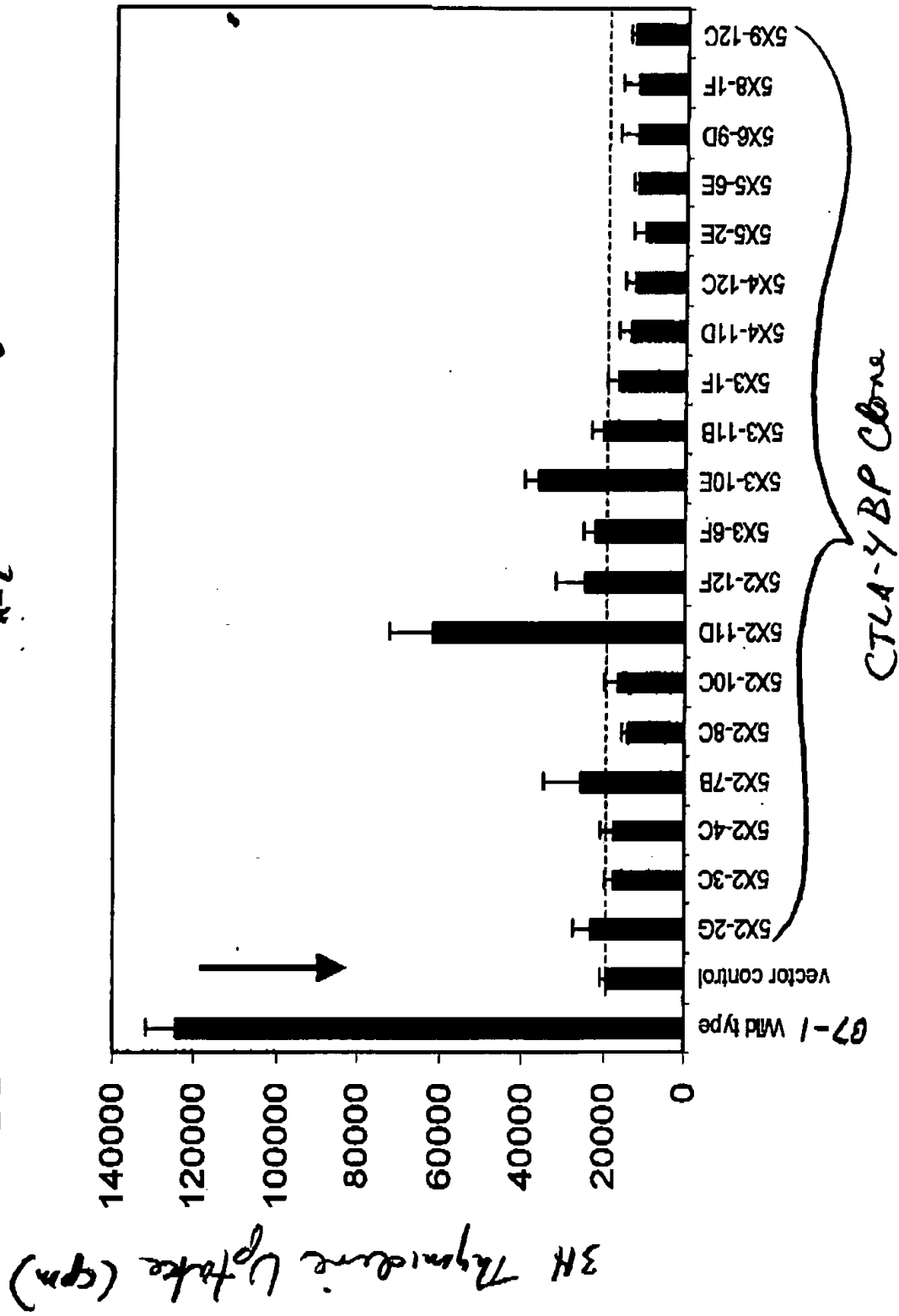


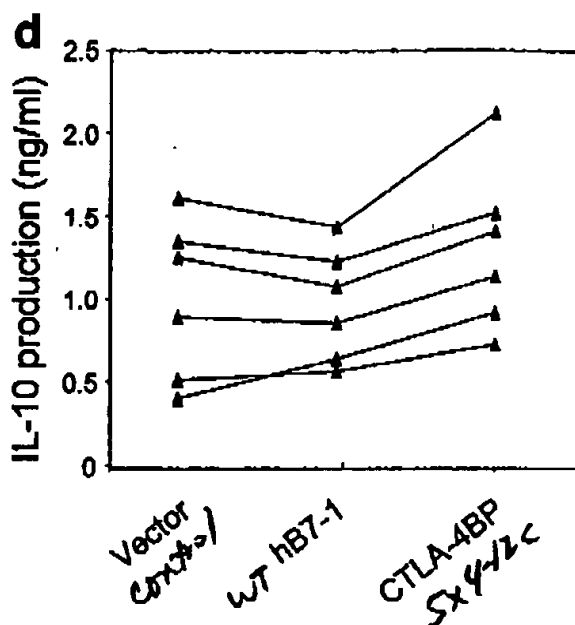
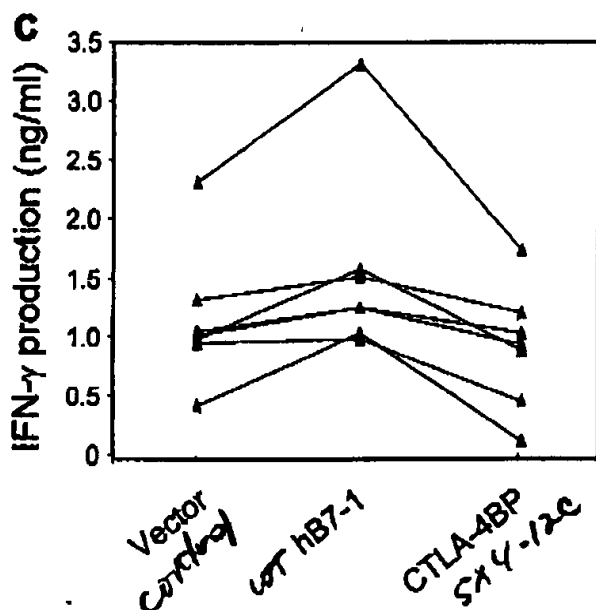
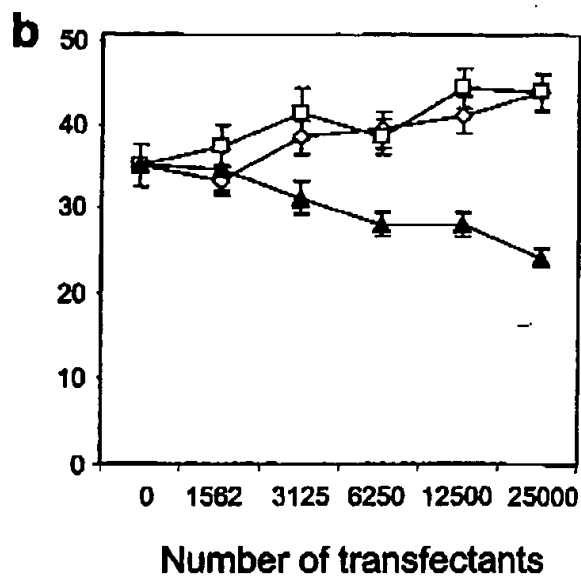
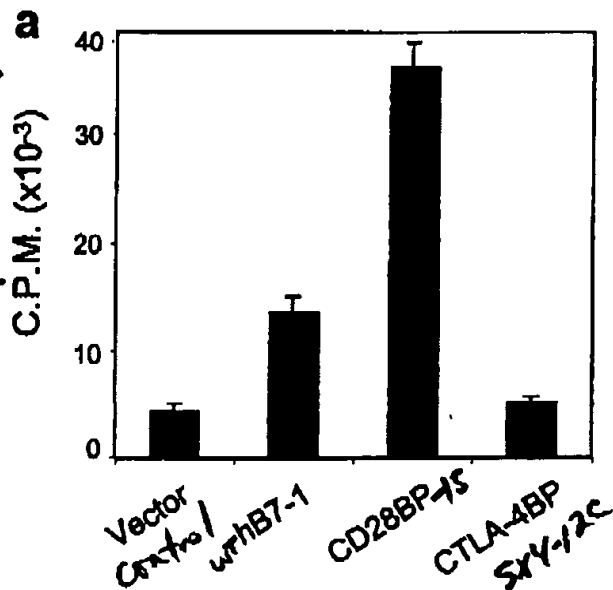
Figure 12

# Suppressed T cell Response by CTLA4BP



Figures 13 A-13D

*<sup>3</sup>H Thymidine Uptake*



102230-12E8860

# Soluble Forms

A

## Human B7.1 sECD

AAAGAPVPYDPLEPPR AHHHHHH



Signal	Extracellular Domain	E-epitope His-tag
( 1 - 34 )	( 35-242 )	( 243-259 ) (260-268)

## B Human B7.1 ECD-Ig Form

BstII hinge -CH2-CH3



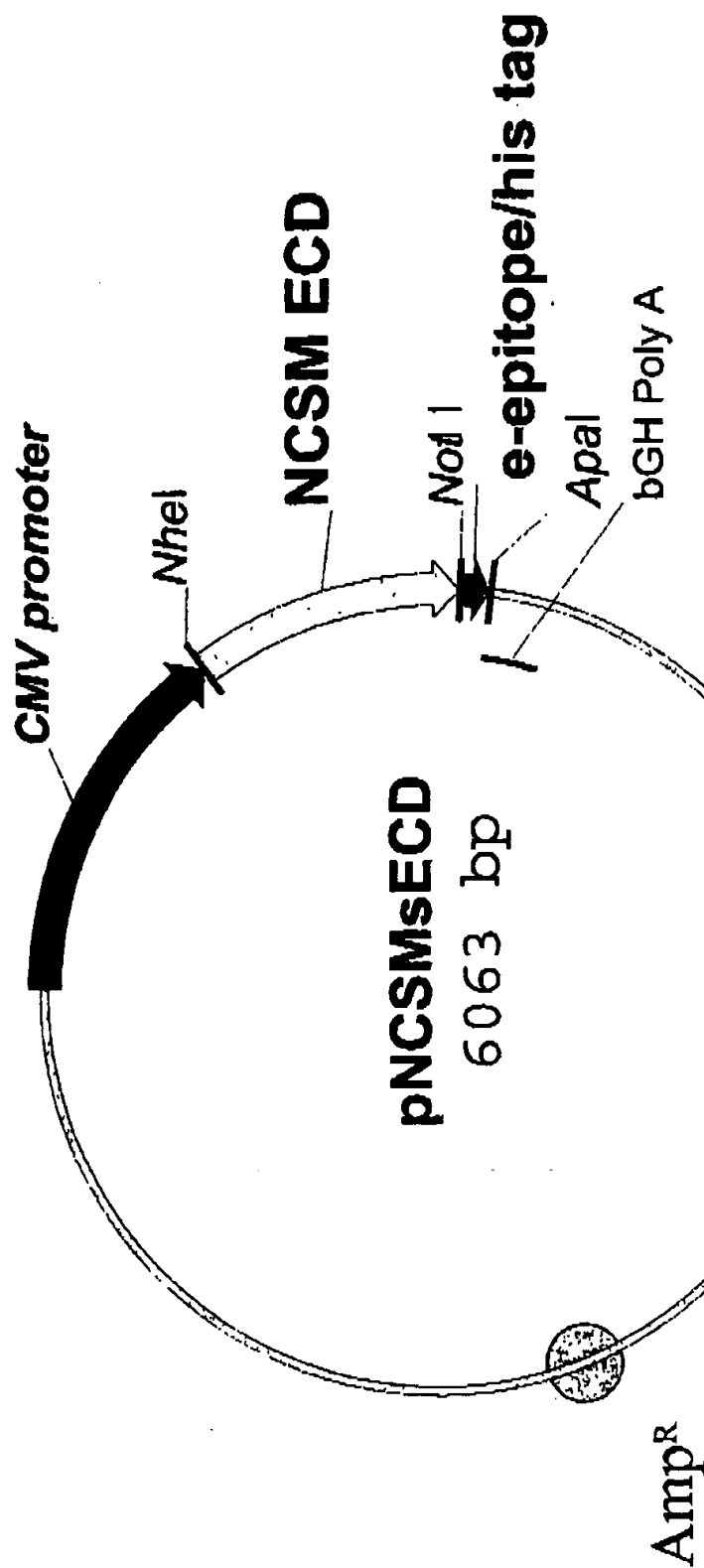
B7.1 ECD (35-242)

Human IgG1 Fc Fragment  
GenBank Acc.# P01857  
Factor X<sub>a</sub>

14A-14B

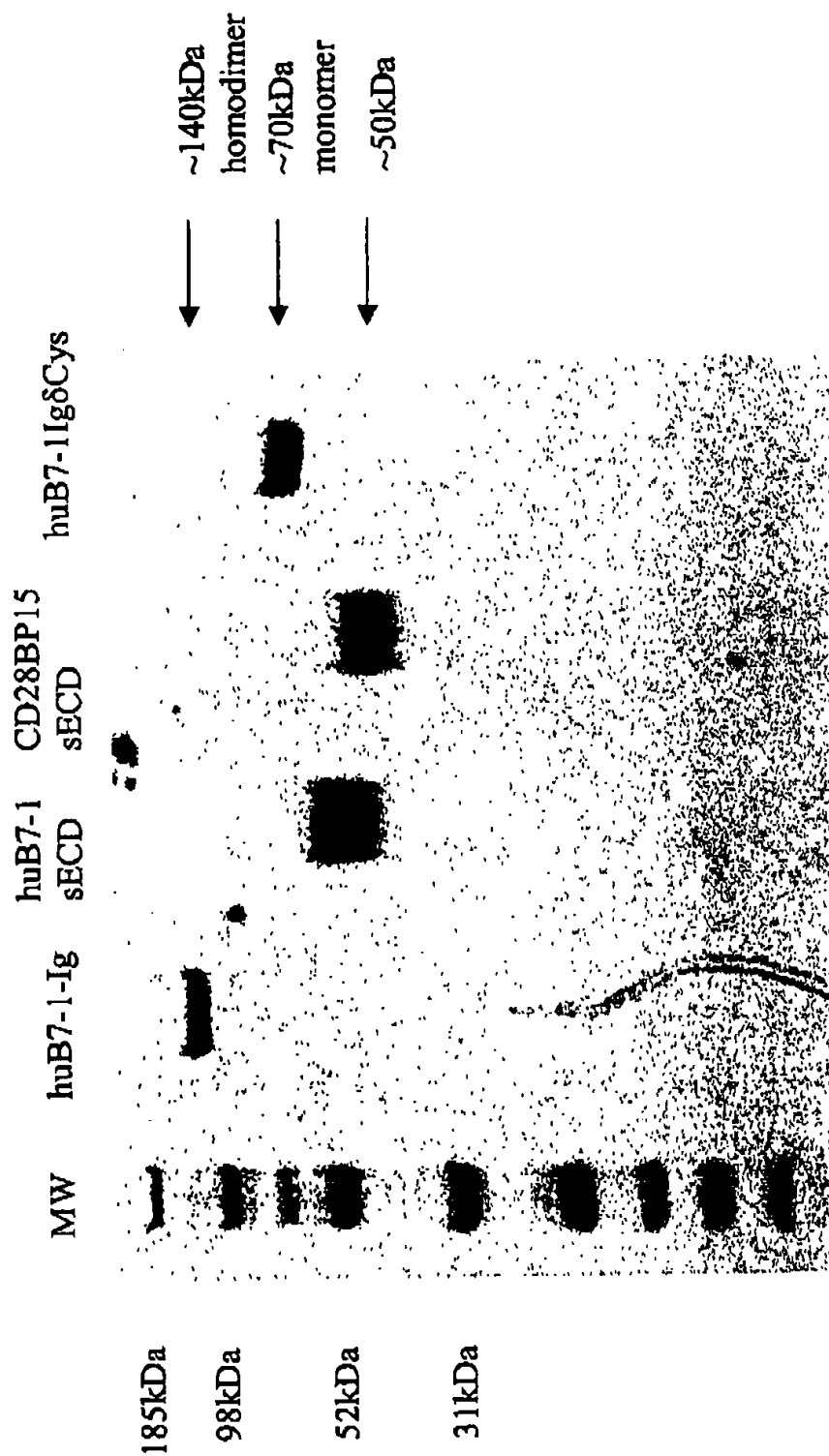
Figures

# NCSM-sECD Expression Construct



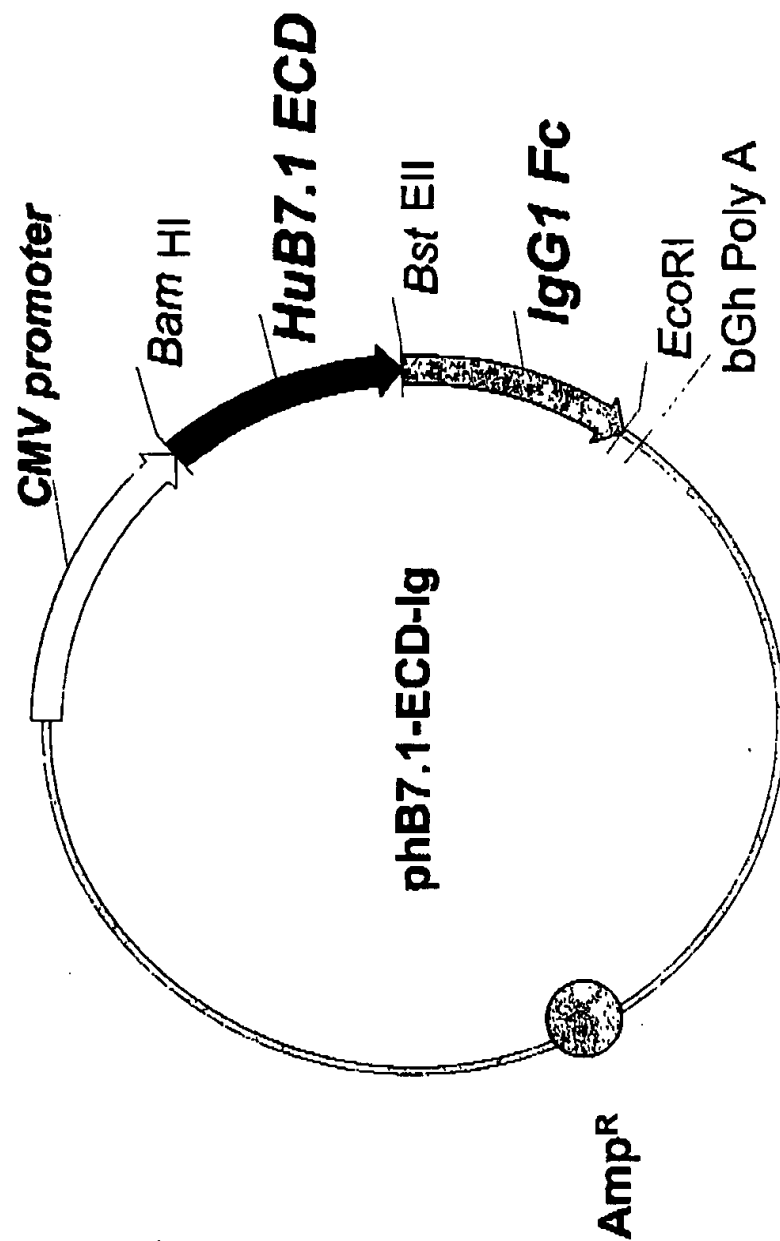
15  
Figure 15

# SDS-PAGE showing various soluble forms of wt & NCSM proteins



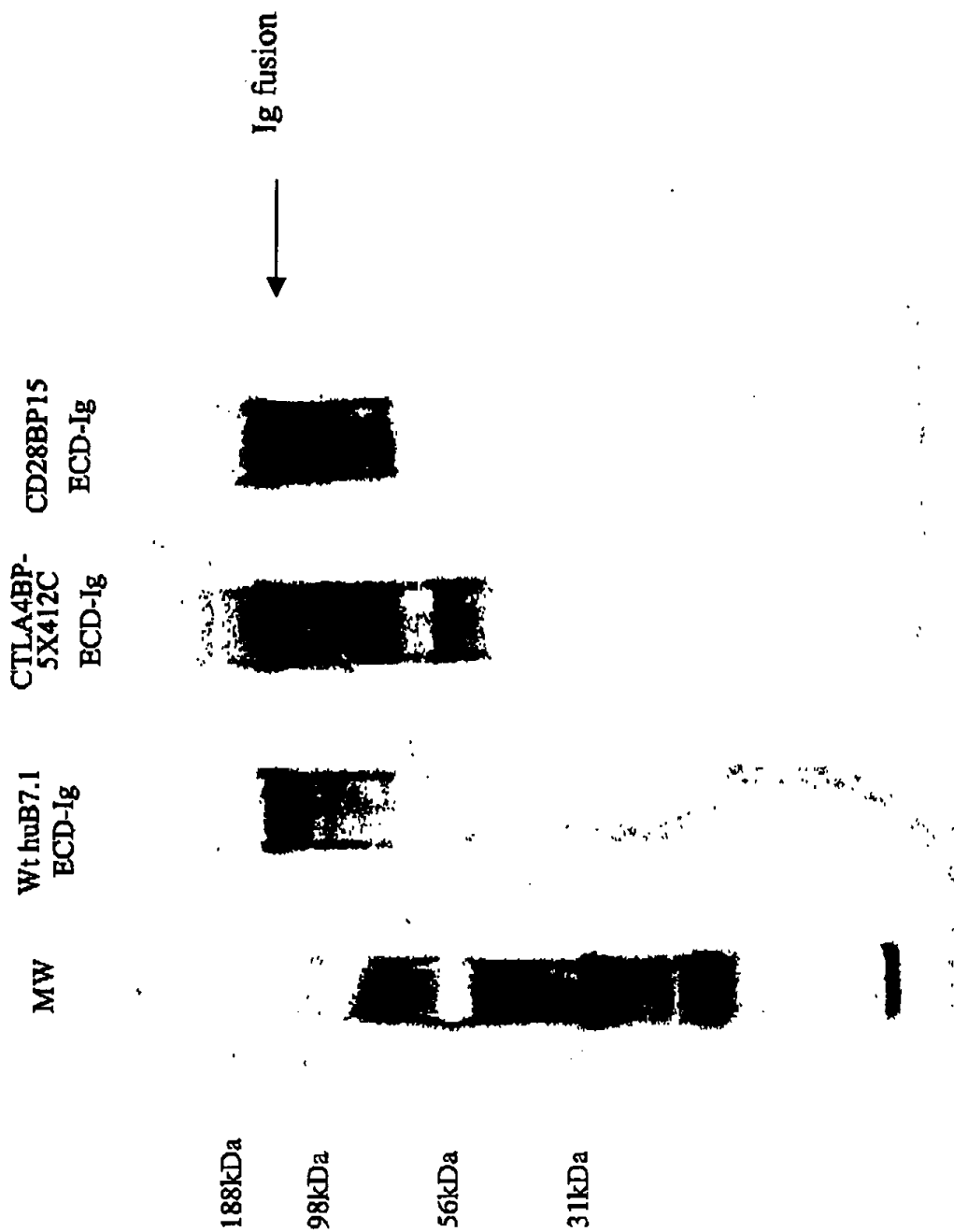
16  
Figure 1

# B7-1-ECD-Ig Fusion Expression Construct





# Scale-up Production of wild-type soluble Human B7.1-, CTLA4BP 5X4-12C-, and CD28BP-15 ECD-Ig Fusion Proteins



# Expression of CTLA-4-BP-Ig and CD28-BP-Ig Proteins

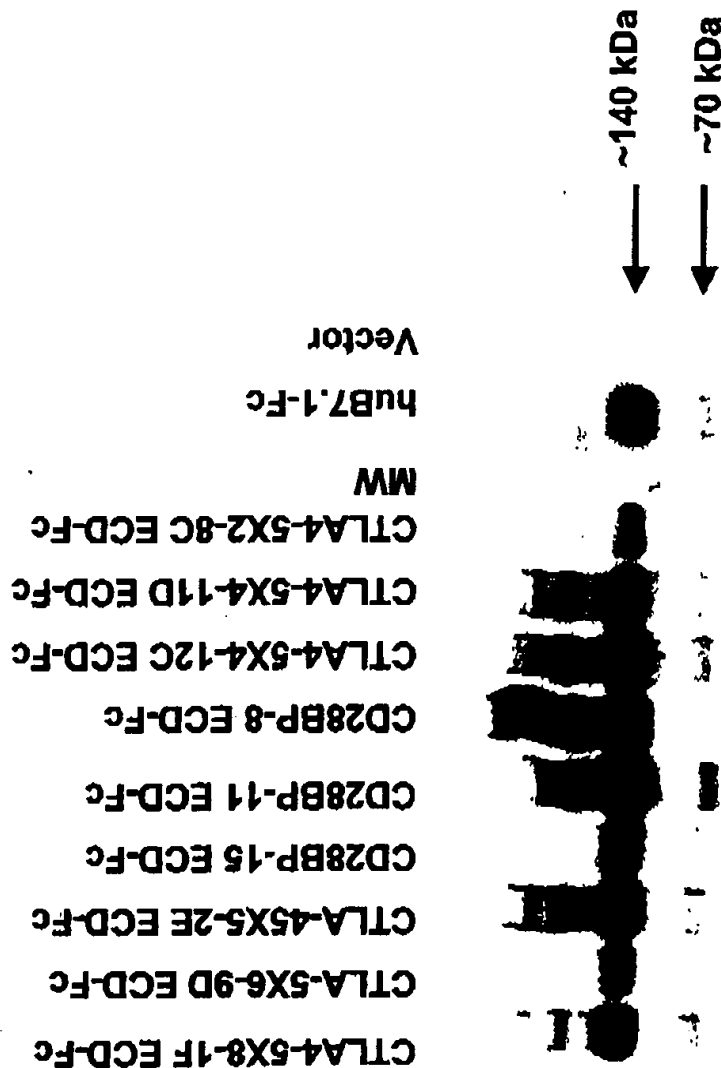
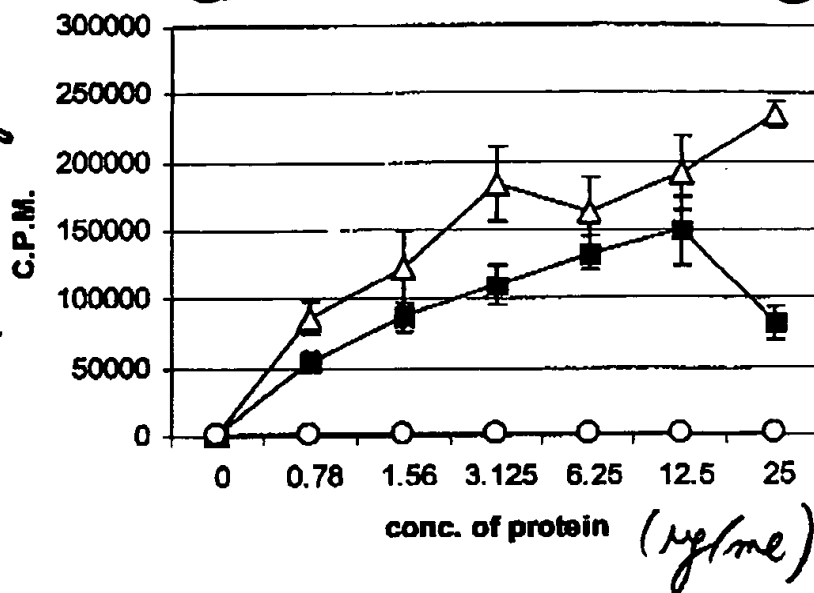


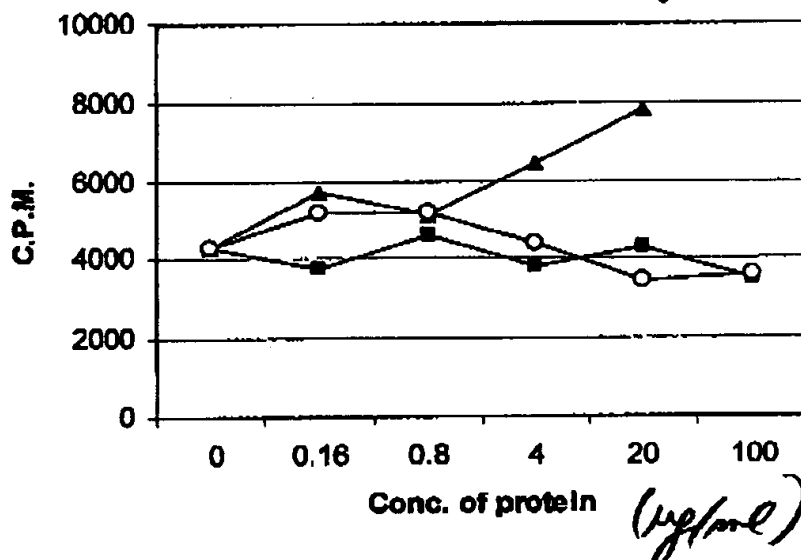
Figure 19

(a)  $^3\text{H}$ -Thymidine Uptake

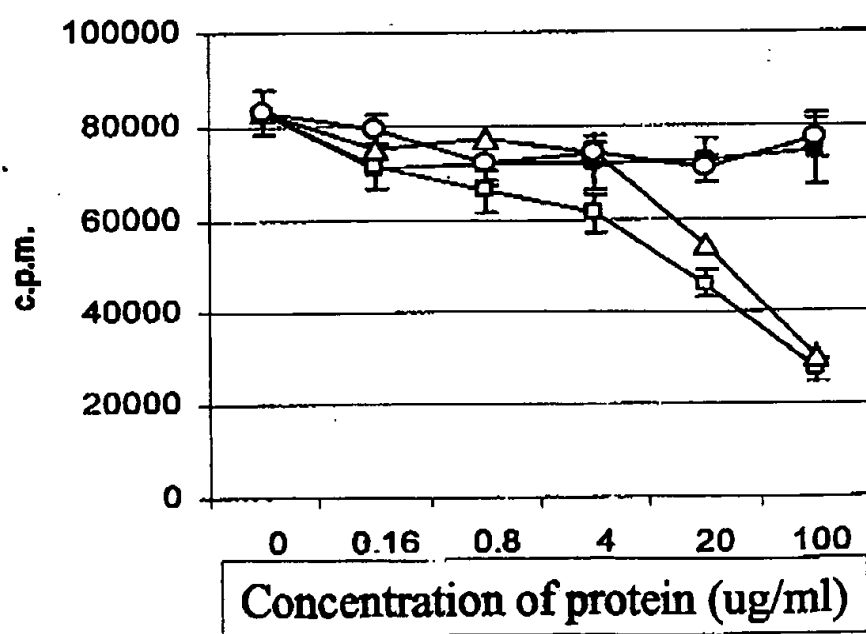


Figures 20A - 20C

(b)  $^3\text{H}$ -Thymidine Uptake

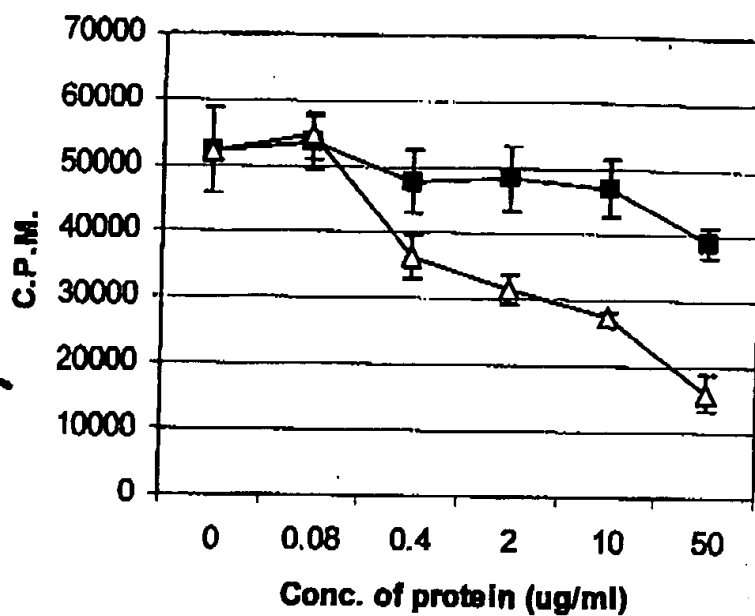


(c)  $^3\text{H}$ -Thymidine Uptake



(d)

*<sup>3</sup>H thymidine  
Uptake*

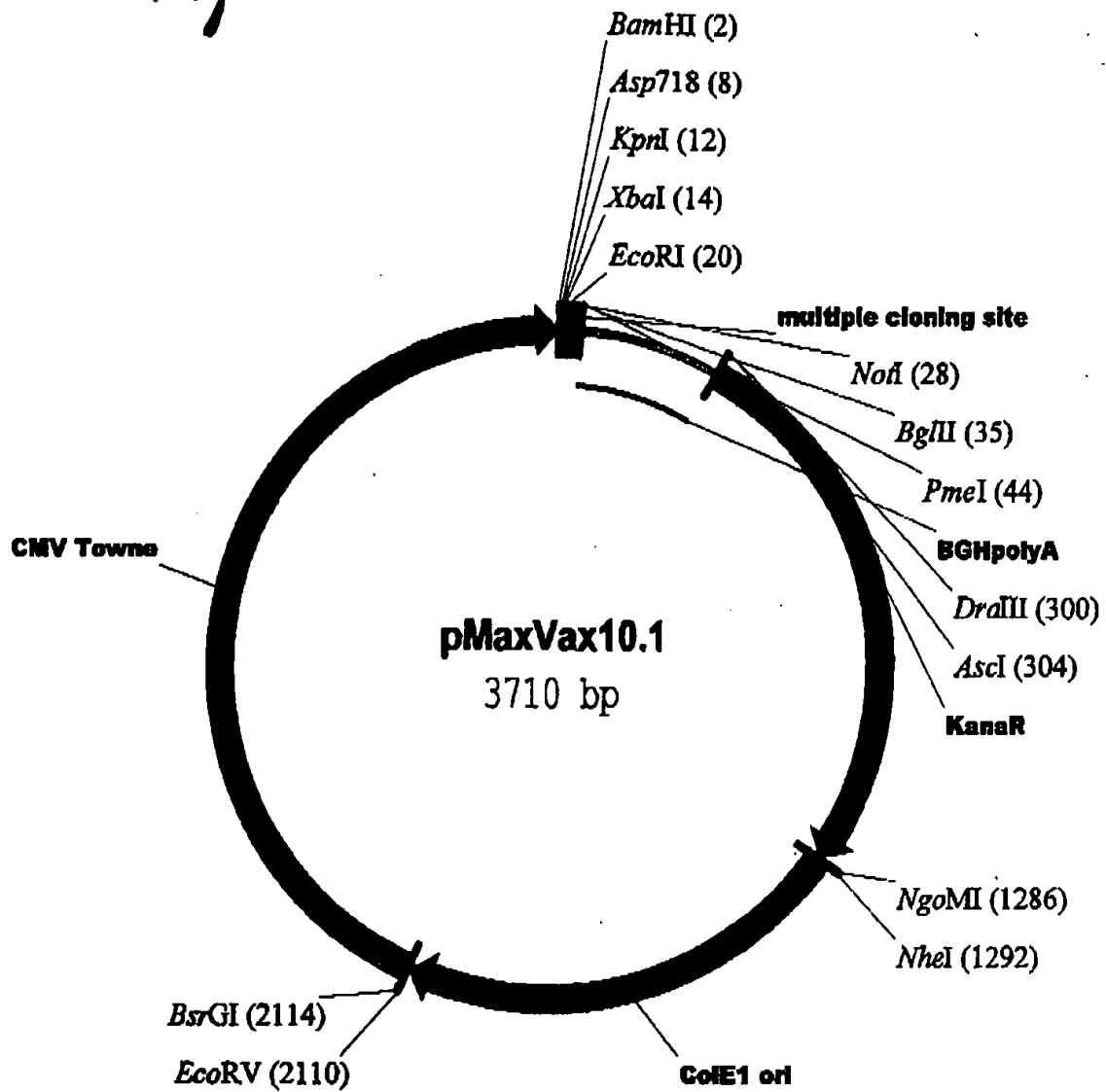


■ Hu B7.1 (ECD)  
△ CD28BP (ECD) *15*

*Figure 20D*

FO2230-42E88860

Figure 21



**pMaxVax10.1**  
3710 bp

Figures 22A-22B

